Novel wood adhesives from bio-based materials and polyketones

Hamarneh, Asal Ismat Mitri

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Asal Ismat Mitri Hamarneh

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Asal Ismat Mitri Hamarneh
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Promotores	: Prof. dr. F. Picchioni
	: Prof. dr. Ir. H.J. Heeres
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to:
Jos, my parents, Rand, Nadi and Rawd

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Chapter 1: Wood adhesives based on natural sources: history and development

Abstract
Since the 1960's, petrochemical based adhesives have replaced natural ones on the world market. However, the need for green products, high environmental standards and the price volatility of petrochemical compounds has led to renewed interest in environmental friendly wood adhesives. However, natural resins still have major drawbacks in terms of product properties (durability and performance) and this limits their application range to mainly indoor applications. This chapter provides an overview of research and development activities aimed at improving the properties of natural based adhesives in order to render them competitive with synthetic adhesives in the market. The review is organized according to a classification of natural wood-adhesives in terms of the renewable source in the formulation: tannins, lignins, carbohydrates, and proteins. Chemical modifications of the natural products as well as blending with petrochemical products are suitable strategies to improve the final adhesive performance. A universal recipe does not yet exist and many solutions still suffer from either very complicated, costly and time consuming modification strategies or from the presence (in the case of blending) of low molecular weight compounds (e.g. formaldehyde) leading to unacceptably high VOC emissions and concerns about health aspects of the volatiles.

1.1 Introduction; the history of wood adhesives

Wood adhesives already have a long tradition [1]. The early Chinese, Egyptian and other advanced civilizations already used natural adhesives in decorative wood veneering and furniture, and musical instrument assembly [2]. Till the beginning of the last century, the dominant adhesives in the market were from animal and vegetable sources [2-5]. As an example, casein glues were used on large scale during World War I to construct the wood-based mainframes of aircrafts. These glues suffered from lack of resistance to moisture and mould growth (i.e. fungi and microorganism attack) [2,3]. This lack of durability limited their use to interior applications only [2,3,6].

The need for better properties led to the development of new adhesives based on petroleum based synthetic resins that showed excellent properties with respect to moist and mould growth resistance. Phenol-formaldehyde (PF) followed by urea-formaldehyde (UF) were the first synthetic resins used as (ply-) wood adhesives. In the 1950s, epoxy resin based adhesives were introduced and offered even better properties. These developments continued into the 1960s and were stimulated by the low prices of the petrochemical-based adhesives. Eventually, this led to the displacement of natural adhesives from the market [2,3,7]. For instance, phenolic and urea formaldehyde resins have replaced blood-, soybean-, and starch-based glues, resorcinol-formaldehyde resins replaced casein-based resins and polyvinyl acetate (PVAc) replaced all collagen-based adhesives [2]. The market composition in the transition period (1960's) is given in *Figure 1.1* [2,8]. About half of the resins are already from synthetic origin. Natural adhesives were mainly produced from carbohydrates and starch.

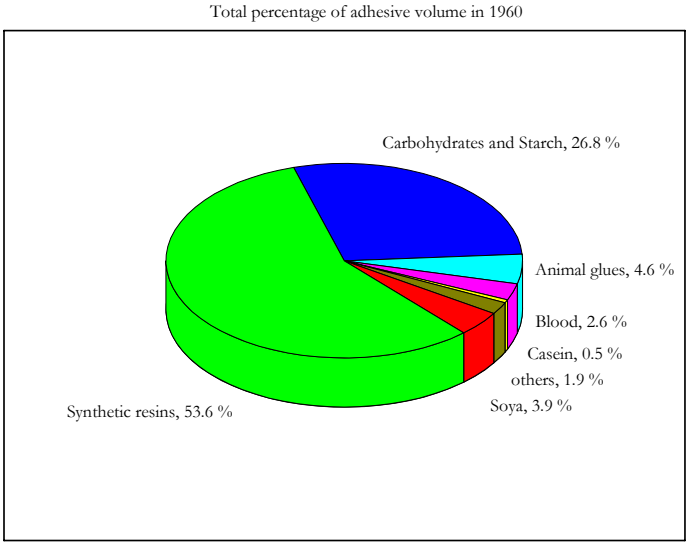

Figure 1.1: Total volume percentage of adhesives in year 1960s [data taken from 9].

The high volatility of oil prices, the growing concern about long-term supply of crude oil and the strict environmental regulations regarding emissions of carcinogenic volatile formaldehyde compounds, have spurred the development of environmentally friendly wood adhesives [10-14]. Materials from natural resources are considered very promising replacements for synthetic resins [11,13,14], provided that suitable solutions are developed to overcome their drawbacks, such as the low moisture resistance [15].

A wood adhesive used for outdoor applications must meet the relevant performance requirements. An example is the European Standard test EN-314. In this test, wood pieces of maple veneers (pre-dried to reduce the moisture content) are uniformly coated with the adhesive, hot-pressed, immersed in boiling water for 72 hours and then cooled in water for at least one hour to room temperature. After this treatment, the strength of the adhesive is determined by tensile test measurements [16].

This chapter provides an overview of the available natural resources for wood adhesive formulations, the technologies and (chemical) modifications required to fulfill the market and environmental demands. Natural products can actually display different roles in wood adhesive formulations e.g. as fillers, co-surfactants, thickening agents. In this work, we focus on the use of natural products as substitutes of the major synthetic (polymeric) components in the formulation. This means that petrochemical additives (e.g. tackifiers and plasticizers) may still be present in the final formulation [17].

1.2 Classification of wood adhesives

Wood adhesives can be classified (*Figure 1.2*) into synthetics and adhesives from natural origin. Synthetic adhesives contain polymers from petrochemical origin. A further distinction of synthetic resins is possible based on their thermal properties. Thermoplastic resins soften and eventually melt when applying heat and solidify upon cooling to room temperature, while thermosetting resins do not soften when heated, often due to the presence of cross-links between the polymer chains due to a curing reaction [6].

Natural adhesives may be subdivided based on their origin, from vegetable or animal source. Vegetable sources are proteins such as soybean, wheat gluten, whey protein, and zein, and carbohydrates such as starch. Examples of animal sources are blood, fish skin extracts, casein (milk), and collagen extracts from animal bones or hides. A typical animal based resin is often dispersed in water prior to the application and then cured by solvent removal or by the addition of a curing agent. The main natural products used for wood adhesive applications are discussed below.

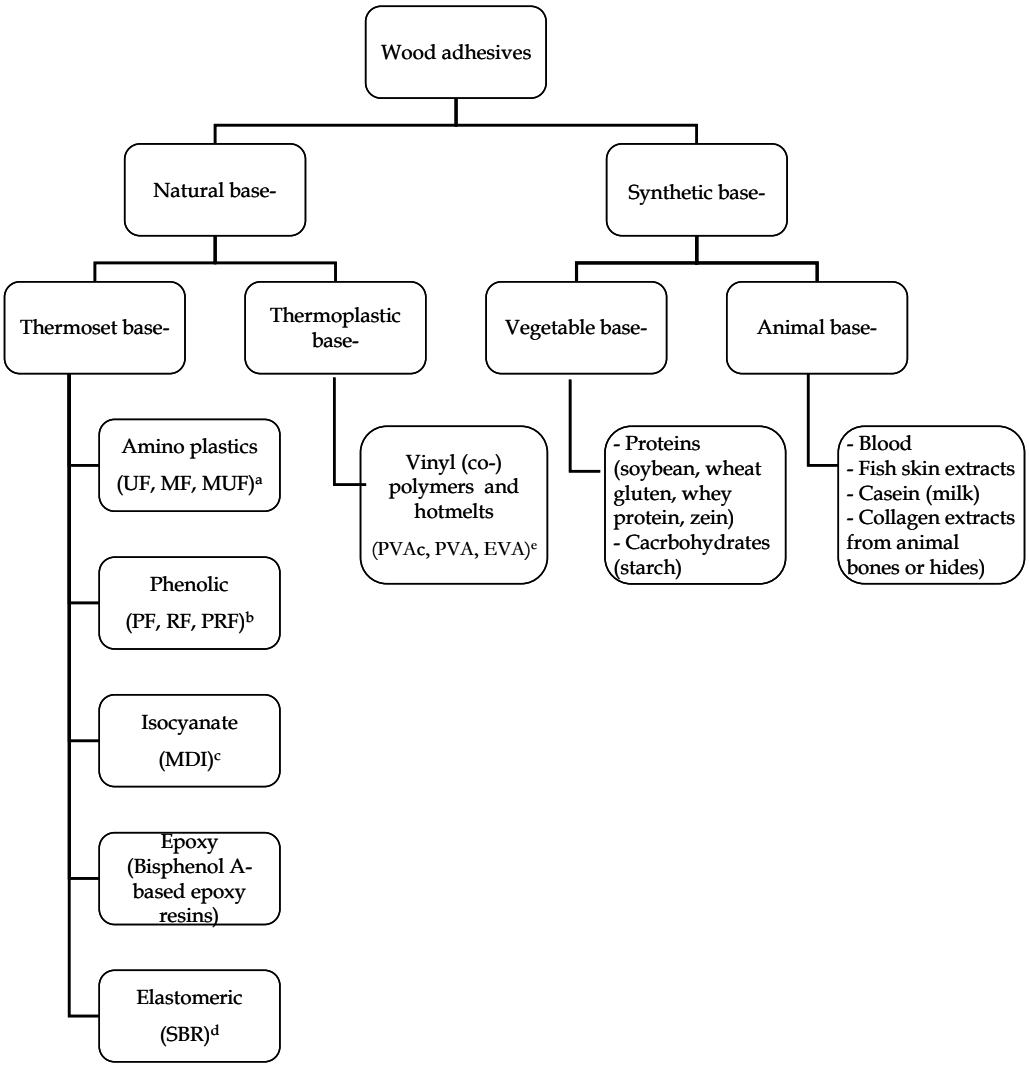

Figure 1.2: Classification of wood adhesives [3,6].

[a]UF: urea formaldehyde, MF: melamine formaldehyde, MUF: melamine urea formaldehyde. [b]PF: phenol formaldehyde, RF: resorcinol formaldehyde, PRF: phenol resorcinol formaldehyde. [c]MDI: diphenylmethane-4,4'-discocyanate. [d]SBR: styrene butadiene rubber. [e]PVAc: polyvinyl acetate, PVA: polyvinyl alcohol, EVA: Ethylene vinyl acetate.

1.3 Wood adhesives based on natural sources

It is widely accepted that the application of adhesives (partially) derived from natural resources requires new approaches and novel technologies for the final product to meet the product specifications for a given application [18]. The product should ideally display the typical advantages of natural materials such as lower toxicity, biodegradability, lower prices, ease of handling, abundance, and a renewable character [6,13,15,18,19]. In the next sections the use of natural products in wood-adhesive formulations will be discussed. The emphasis will be on recently developed modification strategies to improve the final product properties.

1.3.1 Tannins-based wood adhesives

Tannins are natural phenolic-containing materials that are obtained from wood, leaves and fruits by extraction. The extraction is mainly performed in water in a countercurrent process by placing the plant materials in a series of autoclaves. Depending on the original source some additives such as sodium sulfite or sodium carbonate are added to the extraction process. Temperatures of the extraction vary from 70-100 ºC. Typical extraction yields were 33- 43 wt %. The use of organic solvents for extraction (possibly leading to higher yields) in an industrial scale was expensive and caused problems such as pollution and recycling [6,20]. Tannins are often divided in two classes: hydrolysable and condensed tannins (also known as polymeric proanthocyanidins) [6,18,21-24]. Hydrolysable tannins consist of simple phenols (such as pyrogallol and ellagic acid), gallic and digallic acids (*Figure 1.3*). Tannins are targeted for the (partial) replacement of phenol in the wood adhesive formulations.

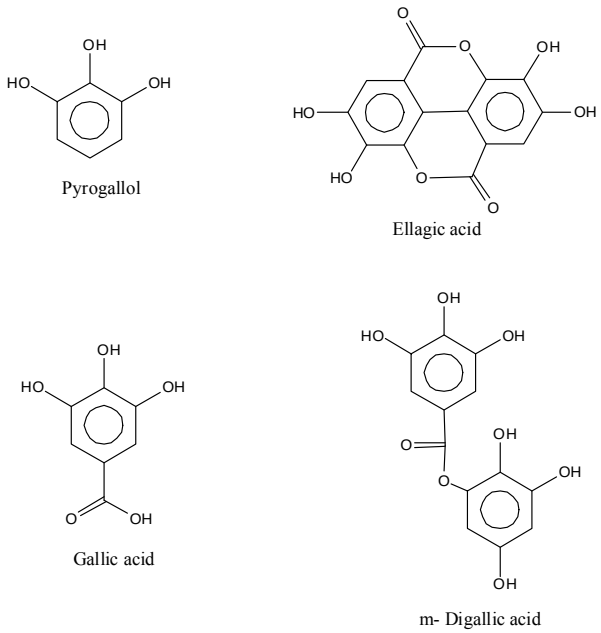

Figure 1.3: Chemical structure of pyrogallol, ellagic acid, gallic acid and the monomer of digallic acid.

However, the worldwide production and the economical interest in hydrolysable tannins is rather low due to their low reactivity towards formaldehyde or other electrophilic compounds needed for the network formation [6,21,22,24].

Condensed tannins comprise more than 90% of the total world production of commercial tannins. They are more of chemical and economical interest for the production as wood adhesives and resins because of their high availability and reactivity [6,21-24]. The reactivity of condensed tannins (*Figure 1.4*) is mainly confined to the A-ring, displaying a chemical reactivity similar to model compounds such as resorcinol and phloroglucinol and to the B-ring with model components such as pyrogallol, catechol [18]. Tannins show a reactivity pattern close to that of resorcinol towards electrophilic compounds such as aldehydes. They react for example with formaldehyde by forming methylene bridges at reactive positions mainly on the A-ring (*Figure 1.4*). However the analogy with resorcinol is limited and the resulting resin properties are entirely different [6,18,20,24]. Instead of multiple additions of formaldehyde to a single aromatic ring, formaldehyde reacts with tannins through single and double additions at different

positions in the rings of resorcinol, pyrogallol, phloroglucinol and catechol in the tannins. This results in differences in the resulting network structure, the cross-linking density and thus in different properties of the final products [6,20,24,25].

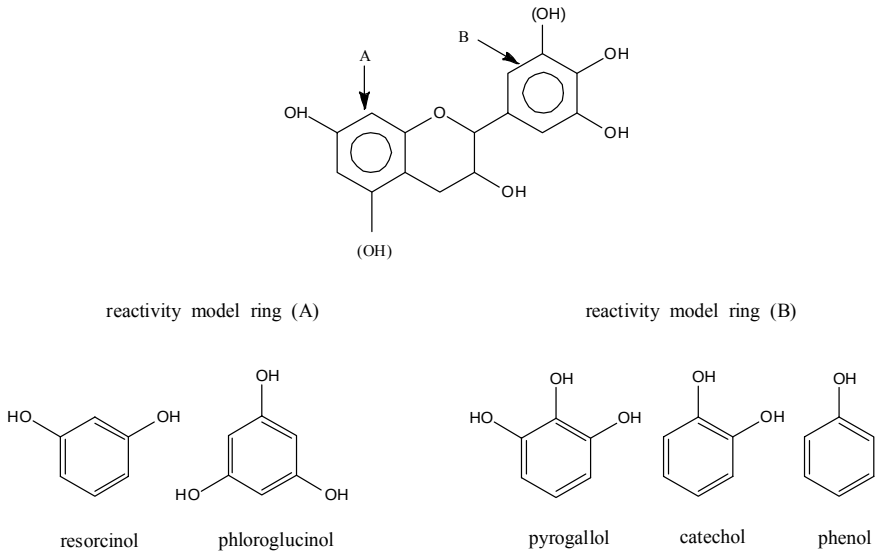

Figure 1.4: Main structural elements of condensed tannins and related model components [redrawn from 21,24].

The main disadvantage of phloroglucinol type is the low yield during extraction and the high reactivity (of the A-ring) towards formaldehyde, which, if uncontrolled, might cause a short pot-life of the glue formulation. Another disadvantage is the corresponding relatively high viscosity of the final water solutions, which might limit their application at the industrial level [18].

Nowadays, the main idea to use tannins is the reduction of toxic free emissions. This goal has been pursued by two approaches [22]. The first consists of the use of hardeners that, in contrast to low molecular weight aldehydes, are not emitted during adhesive preparation and when applying the resin on a wood substrate. Examples of such hardeners are hexamine or methylolated nitroparaffins. For example, when the hexamine decomposes it proceeds to reactive intermediates mainly by the formation of imines and iminoaminomrthylene compounds. These intermediates react with the tannins to form aminomethylene bridges before yielding to formaldehyde. The resulting resins have a long pot-life and satisfy both interior- and exterior-grade specifications [22]. The second method consists of hardening by using the concept of tannin auto-condensation. This reaction is carried out in the absence of external aldehydes and involves opening of the rings under alkaline and acidic conditions followed by condensation [22,24,26-28]-*Figure 1.5*.

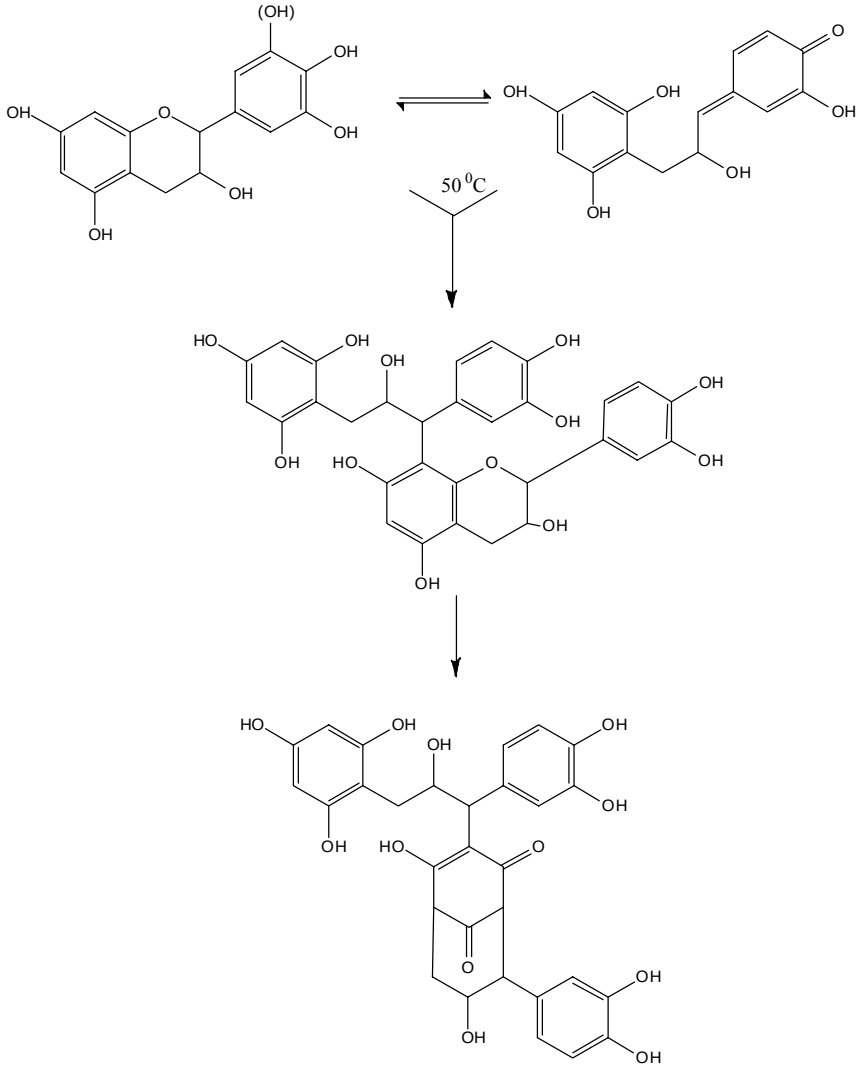

Figure 1.5: Auto-condensation of tannins [redrawn from 26].

Li *et* al [23] have reported the synthesis of a tannin based wood adhesive from condensed tannins in the presence of polyethylenimine (PEI). The tannin-PEI adhesive showed a high shear strength and high water resistance. Unfortunately the tannins-PEI adhesives showed a large loss in the bond strength in long term storage.

Despite the different successful approaches, some general limitations of tannin-based adhesives, such as their high viscosity, still persist. When using them in dilute solutions by addition of water to reduce the viscosity, additional steam will be generated during the curing reaction in the hot press. This results in deformation in the wood elements [29]. Additional disadvantages are their limited availability and supply and, as any natural product, their variable composition, which is always a function of the growing conditions, and renders the corresponding products also variable in their performance [24,25].

1.3.2 Lignin-based wood adhesives

Lignins are three-dimensional polymer networks produced by all vascular plants and are considered the "natural glue" that connects the cellulosic fibers of the plant [18,30]. Lignins consist of phenolic structure elements and they rank next to cellulose in terms of

natural abundance [18,22]. Lignin is formed by oxidative coupling from *p*-hydroxycinnamyl alcohols using hydrogen peroxide and peroxidase [30,31], *Figure 1.6*.

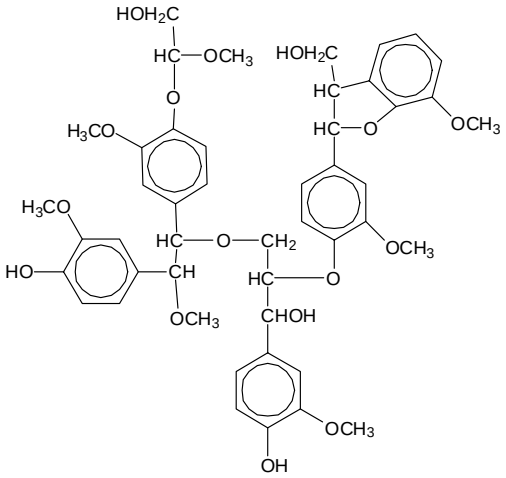

Figure 1.6: Simplified lignin structure.

Lignin is the main by-product of wood pulping to make paper [18,25,30,32]. Lignin displays a relatively low reactivity towards aldehydes or formaldehyde, leading to a reduction in the rate of the hardening process. As a consequence, the press times are much longer than for the state of the art synthetic resins and actually unacceptable for practical applications. This feature has been severely limiting practical applications of lignin-based wood adhesives, even though the research into this class of adhesives dates back to more than 100 years [18,22,32]. At an industrial level, none of the adhesive systems based on pure lignin resins without the addition of synthetic resins or lignin modifications were successful [18,22,32].

Recently the use of thermal conversion methods such as fast pyrolysis and vacuum pyrolysis, pressure liquefaction and phenolysis has been used to produce pyrolytic lignins. They are interesting since the raw materials to produce them (biomass) are abundantly available at low cost; in addition to that it is easy to incorporate it into phenol formaldehyde formulations [32]. Further research is still necessary to evaluate these materials as wood adhesives.

New approaches such as activation of the lignins *in situ* by using enzymes and subsequent application in medium density fiberboard were also not successful [18,22]. Only recently Li *et al* [14] showed that treatment of brown-rot-fungus modified lignin with sodium borohydride ($NaBH_4$) followed by mixing with polyethylenimine (PEI) resulted in a formaldehyde free, strong and water resistant wood adhesive.

The latter are typical examples illustrating that modifications are needed before lignins can be applied successfully in wood adhesive products.

1.3.3 Carbohydrate-based wood adhesives

Carbohydrates in the form of oligomers, monomeric sugars and polysaccharides (from plants sources, microorganisms and exoskeletons of marine animals), have been used as wood adhesives for many years [4,22,33]. They are plentiful, low-cost, easy to apply [9] and they can be used in wood adhesives formulations in many ways [22,33]:

(1) As modifiers for more expensive synthetic adhesives such as PF and UF resins i.e. as thickeners, colloidal stabilizers and flow controllers. According to this approach, it was tried to use carbohydrates, which can vary from glucose to polymeric hemicelluloses, as partial replacement up to 55% PF at laboratory scale under basic

conditions [22,34]. In this case, hemicellulose converts upon acid hydrolysis to different reducing sugars such as xylose, glucose, etc.., while cellulose converts upon acid hydrolysis to glucose [22,35]. These reducing sugars normally can not be used directly as modifiers because they undergo elimination reactions in basic solution to form acidic compounds. These compounds can neutralize the catalyst and therefore preventing the modification of the PF resin. While non-reducing sugars such as sucrose can be used to modify the PF resins, this is considered as a limitation for this type of modification [36].

(2) As source, after degradation, of new adhesive building blocks. This route mainly involves the synthesis of furanic resins. The basic building blocks for these resins are furfuraldehyde and furfuryl alcohol [37] (*Figure 1.7*). Both are accessible by an acidic treatment of lignocellulosic biomass. The C5 sugars react to form furfuraldehyde, which may be catalytically hydrogenated to furfuryl alcohol using heterogeneous catalysts.

Furfuraldehyde Furfuryl alcohol

Figure 1.7: Basic furanic monomers.

Furfuryl alcohol has different applications as a monomer. For example it was used to prepare 2,5-bis(hydroxymethyl furan) through the reaction with formaldehyde (*Figure 1.8*).

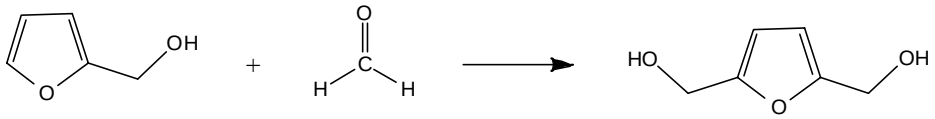

Figure 1.8: Basic reaction to produce 2,5-bis(hydroxymethyl furan) [redrawn from 37].

The 2,5-bis(hydroxymethyl furan) can also be prepared by the hydrogenation of 5-hydroxymethyl furfural (*Figure 1.9*) following the same acid catalyzed process described above.

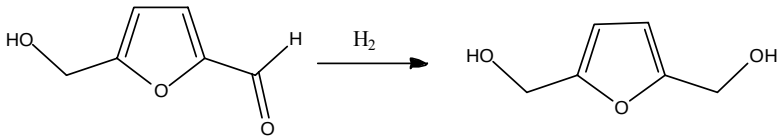

Figure 1.9: Second route to prepare 2,5- bis(hydroxymethyl furan) [redrawn from 37].

Furanic resins are very dark-colored and used in wood adhesives, but they are also very toxic before reaction due to the presence of furfuryl alcohol. This is a serious limitation for such resins to be used as basis for the production of wood adhesives [22,33].

(3) Direct use as wood adhesives. The oldest method for the use of carbohydrates in resin formulations involves dissolution in a strong heated alkali medium followed by cooling to room temperature to prepare wood panel adhesive. After cooling, they are applied as cold press adhesives. The other method was to treat them in acidic media, but the problem with such systems is that the acid used during the formulation degrades the carbohydrates to furan intermediates (see above), which in turn probably polymerize in an uncontrollable way [22]. Recently, research groups have disclosed the

use of liquefied products from cellulosic and lignocellulosic material. They were liquefied in the presence of sulfuric acid under normal pressure using phenol or ethylene glycol. These materials showed good wood adhesive properties [22]. A quite recent development describes a microbial/bacterially derived polysaccharide. These are normally obtained by fermentation of the biomaterial source. In this case no volatile organic compounds (VOCs) or toxic chemicals are formed. These polysaccharides displayed comparable shear strength to the commercial PVA-based adhesives with maple substrates at high humidity [38].
Independently of the role of the carbohydrates in the adhesive formulation, the main disadvantage coupled with their use is that the corresponding "bond strength" with the wood surface remains, even after application on the wood surface, sensitive to water. Moreover, some authors [4,22,33,34] claimed that in general carbohydrates show slow curing rates, which then demands relatively longer pressing times. Finally, they are relatively expensive, thus rendering the competition with the existing formulations even more difficult from an industrial point of view. It is clear however that also in this case modifications of the base compounds are needed to overcome these disadvantages for future applications [4,22].

1.3.4 Protein-based wood adhesives

1.3.4.1 Animal-base wood adhesives

Wood adhesives based on animal sources are known in history as "traditional" ones [5,9,39]. They are obtained mainly from cattle and from the hydrolysis of protein collagen of animal hides and bones [4,9,39,40].
Hide glues have higher molecular weight and are therefore stronger than bone-based ones. The hides are usually treated with acid and then cooked. The extract is filtered, dried and then finally ground. The process of obtaining bone glues comprises the treatment of bones with steam cycles under pressure and then extraction with hot water. The liquors are then filtered, centrifuged in order to remove fats, dried and then ground [9]. An important property of animal adhesives, which made them easily available even not in an emulsion form, is the fact that that they are able to form gels, which involves intra- and inter-molecular rearrangements upon cooling, thus providing an immediate, strong bond. Further drying can provide final resilient strong bonds with the wood surface [5,9,39,40]. Another property of the animal glues besides their bonding characteristic is their film forming property. The latter is a consequence of the use of these materials in emulsions rather than in gel form. However, in the former case (i.e. emulsions), these proteins can not be used as such, but need a modification strategy in order to improve the final adhesive properties. This was mainly achieved by blending them with different chemicals such as glycerine, sorbitol, and other glycols, sometimes with mineral fillers to increase the film flexibility property. Afterwards they were graded according to the viscosity and the gel strength [9,39,40].
Animal glues found also many applications other than wood adhesives, such as bookbinding, paper manufacturing, gummed tape, cork composition, match heads, and in sandpaper manufacturing to bind the silicon, aluminum oxide emery and abrasive rings [9,39,40].
Among all possible animal-based wood adhesives, the ones based on the use of animal blood and caseins represent the most common examples. These will be discussed in the following paragraphs.

The use of animal blood-base adhesives represents one of the improvements of practices of antiquity [5,39,40] . At first, they were used in the liquid state; however, the fast spoilage rate of the liquid blood generated a limitation for the availability and usage of such adhesives. The main techniques aimed at the use of these materials in adhesives were based on the drying process of the blood without loosing the water solubility i.e. no protein denaturation, collecting it in commercial quantities and finally storing it for use [2,8,39,40]. The most used sources for dry blood glues are from steer, hog, and beef. The proteins included in the different animal bloods are typically serum albumin and globulin and even the red cell hemoglobin [2,9,40]. Because of the instability of the fibrin in the solution, the fibrin-clotting component is removed at the end of the drying process, mainly by spray drying, agitation or acidification pre-drying. The dried blood is then 100% active protein adhesive. Some chemical denaturants can be added to the blood solution before the drying process to modify the adhesion properties [2,6,8].
However, in order to be able to control the adhesive performance some physical properties of the blood glues such as viscosity, solubility (by adjustment of the spray drying conditions), and dispersability in alkaline solution should be finely controlled [2,9,40]. Blood-based glues use most conventional methods of spreading on the wood surfaces, including roller, knife and extrusion [2]. Blood proteins, as the rest of proteins-based adhesives, are usually formulated by dissolving the blood in water, followed by dispersion and treatment with an alkaline agent such as hydrated lime or ammonia or caustic soda. The major advantage of this process lays in the enhanced heat resistance of the corresponding adhesive as well as reduced hot-time press [2,6,41]. First useful attempts in the plywood industry were tried by reacting formaldehyde vapor with powdered blood with controlled water solubility. The resulting final adhesives were tunable in terms of viscosity [42]. Blood proteins were also applied as foaming agents in PF resins for the plywood industry. The foamed PF adhesive or "air-extended PF adhesive" can be extruded onto the wood instead of being sprayed. In this case it reduced the costs of the PF adhesives used [2,6]. Breyer *et al* have described [43] other blends of UF resins modified with the addition of a blood protein, with a description of the function of the proteins as binding-enhancer. In order to reduce the formaldehyde emissions in the environment other kinds of blends have been tested. These blends included sprayed-dried animal blood with soy flour and had given ideal adhesive properties for the wood industry. In this case, the blood provided water resistance and rapid hot cure, while soy flour provided the granular consistency for machine applications. This blending reduced the costs, as adhesives based only on blood were very expensive [19]. It should be noticed that in recent years much thoughts were devoted to the handling or inhaling blood particles, possibly containing diseases, which might further hinder the use of these proteins at a commercial level [5,44,45]. Another option in this respect is constituted by proteins that are not directly extracted from blood, such as casein. The latter is the major protein of milk; it is precipitated/recovered from skim milk (buttermilk) by acidifying the milk to pH 4.5. This is achieved by adding acid to the milk or by allowing the milk to sour naturally [2-6,8,9,40]. To obtain casein protein the curd must be first washed with hot or cold water, dried and then ground [2,9]. In the same manner as blood proteins, the casein is mixed with simple alkali such as lime or sodium hydroxide in proper proportions to produce casein adhesives. Finally, water is only added at the time of use [2,6,9]. When using caseins for adhesive purposes, the particle size of the casein ground must be carefully controlled in ranges between 250-500 μm. Sizes above the 500 μm may not dissolve/disperse totally during the following step of adhesive preparation, while sizes less than 250 μm tend to form lumps on wetting [2,8]. Casein adhesives, contrary to blood adhesives, can provide the property of water resistance [2,8,40], usually achieved by controlling the lime content [2,9]. Excess of lime

ensures water resistance but decreases the pot-life, while less lime content provides longer pot-life but reduces the water resistance. Commercial casein adhesive formulations balance the lime content to values between 15-25 % based on dry casein weight [2,8,9]. It is known that the maximum adhesive ability of casein, as in blood and soy adhesives, is achieved by complete dispersion of folded proteins in an alkaline salt medium. Since sodium hydroxide cannot be combined in a dry formulation, a decomposition reaction between calcium hydroxide and less alkaline salts, such as sodium fluoride or sodium carbonate, is performed and the produced residues are insoluble calcium compounds [2,8]. The viscosity of such adhesives can be controlled by adding thickeners such as sodium sulfate, or thinners such as sodium sulfite or sugars, fillers, nondrying oils, etc... In combination with this, the mold resistance for exterior and interior applications should be provided by adding fungicides (preservatives) to the mentioned casein formulations [2,4,8,9,40]. In this respect, blends of casein with soy flour have been prepared. In this case, the casein provided deep bonding, heat resistance, short clamping cycle cold cure and stickiness consistency (tackiness), whereas soy flour gave granular consistency which helps in quick loss of water [2,44]. Another unique property of casein adhesives is their fire resistance, while all other adhesives mentioned previously burn to char and loose the bonding strength, casein adhesives showed durability in this respect [2,8]. It should be also mentioned that the price of casein depends directly on the supply/demand economics of milk products rise and fall, since the casein in this case competes with the worldwide food usage of milk protein and products [2]. Beside wood adhesives, casein can be applied in other areas such as paper sizing and bonding, chipboard laminating, or for label adhesives for beer and soft drink bottles, because it has good optical properties [2,4,9,46]. In addition to that, ammoniacal casein can be also blended with styrene-butadiene (SBR) rubbers or neoprene latexes to obtain adhesives useful in bonding aluminum foil to paper [9].

1.3.4.2 Vegetable-based wood adhesives

1.3.4.2.1 Soy protein-based wood adhesives

Soybean consists mainly of protein, oil, carbohydrate, ash and minerals [9,11,40,47]. The protein content is dependent on the source/grade and may vary from 30-55 % based on oil-free basis, the carbohydrate content ranges from 30-34 %, the oil content from 15-24 %, and the moisture content after processing is less than 10 % [2,11,40,47]. In order to get these useful products from the soybeans, the beans are first dehulled, milled at high pressure and after that undergo solvent extraction to remove the unwanted oil [2,8,48-51]. Heat and humidity are very important factors in the protein extraction from the soybeans. For example, for adhesive purposes the process temperature for the soybean meal should be below 70 ºC to preserve the alkaline solubility of the protein [2,16,48]. Soy proteins are attractive in adhesive formulations because of their unique properties such as the ease in handling, renewability, ability to process at hot and cold temperatures, biodegradability, and being abundant. However, besides the variety of chemical groups already present on the protein (e.g. amines, carboxylic acids, hydroxyls and mercaptans), further modification studies and efforts have been devoted to improve the adhesion strength, reduce the cost and to improve the water resistance [49,52-54]. Treatment with soluble strong alkaline such as sodium hydroxide, trisodium phosphate, etc.. is necessary to expose and disperse more amide functional groups to maximize the adhesion. This relays on the concept that by breaking the internal hydrogen bonds in the coiled protein molecules (unfolding), the polypeptide chains become more available for adhesion to the

wood surface [2,19,49,55,56]. It should be mentioned that even the strong alkaline soybeans glues are almost colorless when applied as a film. However they cause reddish-brown color stain on the wood surfaces as they cure, which could be seen as a disadvantage of such adhesives [2,8,9,19]. Other disadvantages are short pot-life, long press time, poor biological stability e.g. mold resistance, and most important, poor water resistance which limited their usage just to interior application [2,49]. Slight improvement of the product performance with respect to these disadvantages can be booked by addition of hydrated lime, copper salts, rosin and sodium silicate to the above-mentioned high alkaline mixture. This can help in maintaining an appropriate level of viscosity for longer adhesive working life, and improve the water resistance if the glue cures [2,57]. Moreover, soybean proteins can be protected from fungal attack by adding chlorinated phenols such as pentachlorophenol or ortho phenylphenol, or copper-8-quinolinolate and copper naphthenate [2,4,8,9,19].

Furthermore, in order to improve the water resistance, working life and the dispersity/consistency of the protein, some cross-linking agents and denaturants are added such as sulfur compounds. Examples are carbon disulfide, thiourea, zinc sulfate heptahydrate, inorganic complexing salts and formaldehyde donors such as dimethylolurea, sodium formaldehyde bisulfite, hexamethylenetetramine, and trimethylolphenol, defoamer such as pine oil [2,4,8,9,19,52].

A different approach towards the performance improvement is constituted by the chemical modification of the original peptidic chains. In this respect, different enzymes such as papain, trypsin, chemotrypsin, pepsin and urease have been used to modify the soy protein isolate or concentrate. The adhesive strength and viscosity of the resulting modified adhesives were comparable with the commercial ones. The advantages of enzyme modification are: mild conditions, high reaction rates and specificity [19,58]. The high costs of the enzymes and the difficulty to separate them after the modification still count as a disadvantage. In addition to that, increasing the reaction curing time tended to decrease the shear strength [19]. Guanidine hydrochloride (GuHCl) was used to modify the soy protein isolate, but the shear strength of the obtained adhesive had decreased after water soaking which limited such adhesives only to interior application [11,19,53,55,59]. Urea and sodium dodecyl sulfate (SDS) and sodium dodecylbenzene sulfonate (SDBS) have also been used to modify the soy protein [19,43]. Another modification is achieved by imparting the soy protein with functional groups from marine adhesives, thus practically converting the soy protein to a water resistant wood adhesive [13,14,60,61]. The first research attempting at such modification was performed by grafting of dopamine (DA) to soy protein isolate (SPI) via an amide linkage (*Figure 1.10-(I)*). The amount of phenolic functional groups affected significantly the shear strengths and the water-resistance [13,61].

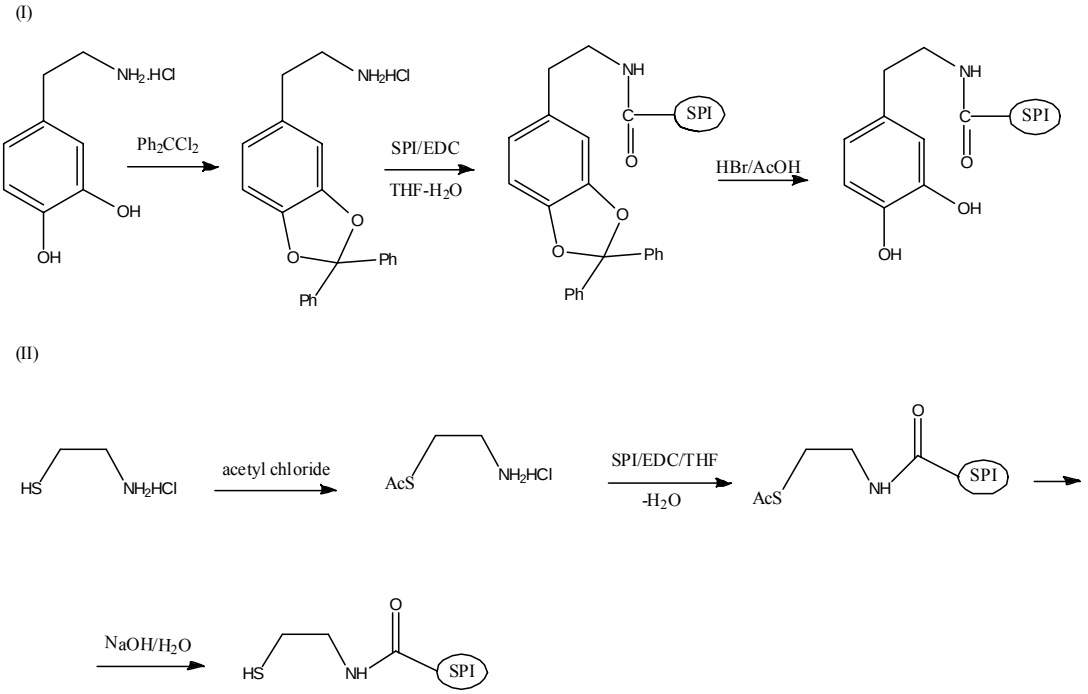

Figure 1.10: Preparation of (I) dopamine-grafted soy protein isolates (SPI-DA), (II) cysteine-grafted soy protein isolate (SPIs), EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride [redrawn from 13,6].

Another study was carried out by grafting cysteamine to soy protein isolate by amide linkage, (*Figure 1.10-(II) and (III)*). It was found that increasing the amounts of the (–SH) groups increased the shear strengths and improved the water resistance [60,61]. However from all the previous attempts it is known that, despite the fact the produced modified soy protein has an improved water resistance and strong bonds with the wood surface, the proposed modifications are rather difficult (e.g. they require relatively long reaction times) from a chemical point of view as well as being costly [13,14,60,61]. A different modification path consists in the soy protein treatment with lignin in a presence of a curing agent based on amine, imine, or nitrogen-containing heterocyclic functional group and a boron-containing compound as a cross-linking agent. In this composition the curing agent has a dual functionality, in providing curing and functional groups that would react with the soy protein to make wood adhesive with strong bond strength [62]. The role of the modified protein adhesives as for the pure natural protein adhesives is that the modified ones have the ability to disperse and thus partially unfold in solution and to interact with the adherent via non-polar and polar groups in the protein *(Figure 1.11)*. The unfolded molecules will increase the contact area and bonding with the adherent surfaces during the curing process and therefore increasing the bond strength [55,58,63,64]. The viscosity of the adhesive is important because it is the result of intermolecular interactions from the disulfide bonding and the electrostatic interaction in the protein molecules. The moment the contact with the wood is achieved the hydrogen bonds can be formed between the hydroxyl groups from the cellulose and the oxygen or nitrogen atoms from the protein and hence chemical bonds are formed [19,56,65,66].

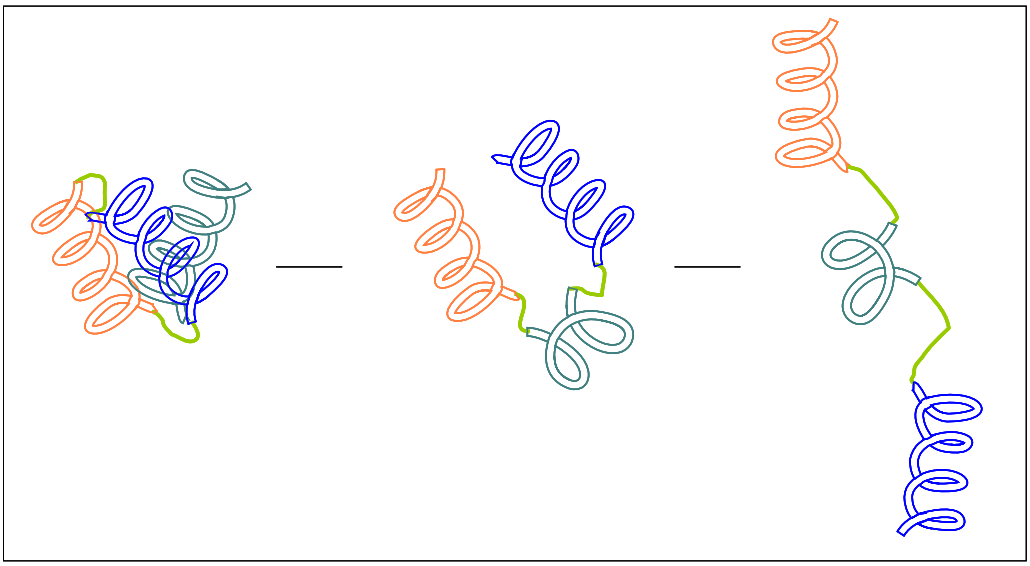

Figure 1.11: Unfolding of protein [redrawn from 67].

Enhancing the adhesive performance can be achieved by forming blends of soy protein with other natural (proteins) or synthetic resins adhesives. These formulated blends have incorporated/promoted the good properties from both components to get new useful adhesive material [2,19,49]. Blends of soybean with formaldehyde, phenol formaldehyde, melamine formaldehyde, phenol resorcinol formaldehyde have been investigated in the literature. The following examples are listed below [2,4,19,43,68,69]:

Urea-formaldehyde-soy blends: such blends gave faster cure time, and enhanced the shear strength with lower formaldehyde emission. The major drawback of these adhesives is the use of petroleum-based raw material, which is a non-renewable source if compared to the soy protein and the emission of formaldehyde is still present [43].

Formaldehyde-soy blends: in these blends soy protein is denaturized, modified and stabilized with aldehydes. The obtained protein could be copolymerized with sources of formaldehyde resins such as phenol formaldehyde, urea-formaldehyde and melamine formaldehyde via methylene linkage. More specific, the hydroxymethyl group in the proteins can condense with either the hydroxyl methylol group in the phenol or with the hydrogen of phenol, both lead to form a stable N-CH_2-phenol linkage. Copolymerization could be possible between two protein hydroxylmethyl groups to yield a protein-CH_2-protein methylene linkage (*Figure 1.12*) [68].

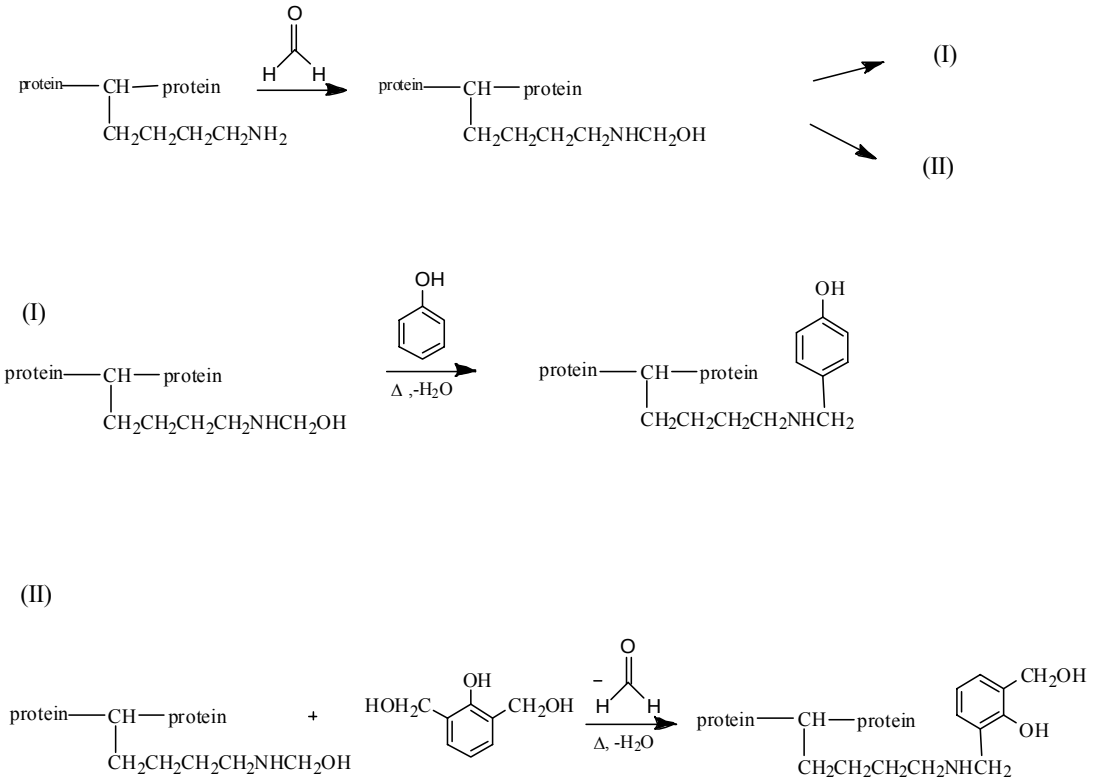

Figure 1.12: Examples of protein functionalization for improved compatibility with urea-formaldehyde resins (UF resins) [redrawn from 68].

The phenol formaldehyde resin could be used as a cross-linking agent, where the hydroxymethylol group from the cross-linking agent was reacted with the hydroxyl groups in the soy protein to form methylene or ether linkage [70]. The disadvantage of these adhesives is the use of the phenol and formaldehyde as a starting material. Using these materials will still emit formaldehyde and phenol to the environment during processing and curing, even if these amounts are lowered in this type of adhesive.

Soy protein can be also blended with natural material such as casein and blood some examples are listed below [2,19]:
Soybean-Blood adhesives: blends with blood adhesives were by far the most extensively hot-pressed adhesives until 1960s. In such blends, blood provides water resistance and short hot cure time while the soy provides granular consistency [2,5,8,19]. Still the disadvantage of using such adhesives lies in the fact that handing or inhaling blood particles might contain possible diseases [5,44,45].
Soybean-Casein adhesives: in these blends, casein provides deep bond, tackiness, cold cure and heat resistance while soy provides granular consistency, which helps to dissolve quickly in water. Such blends maintain good adhesion properties even in fire. These blends differ from the rest of the mentioned adhesives above in that all the ingredients are dry-blended into a single composition and water is added only to prepare [2,5,8,19].
Finally, it is worth mentioning that soy protein together with other adhesives have been applied not only in the wood adhesive field but also in different fields. Some applications include: soy plastics [19,71,72] paper coating composites [73-75], tissue engineering for medical uses [76], and labeling adhesives [77].

1.4 New environmentally friendly synthetic wood adhesives based on polyketone

An urgent need to new environmentally friendly wood adhesives was due to the new regulations and environmental concerns regarding the emissions of formaldehyde and its carcinogenic vapor, and due to the limited amounts of petroleum sources [13-15,19].
Aqueous polymeric emulsions were prepared in one-pot reaction from the chemical modification of the alternating thermosetting polyketone using 1,2-Diaminopropane (1, 2-DAP) via the Paal-Knorr reaction pathway [16,78]. The obtained polymeric amine acted as a surfactant upon protonation and is used for the self-emulsification of the polyketones to obtain wood adhesive emulsions [16]. The emulsions were successfully qualified as wood adhesives according to the European Standard (EN-314) with high water resistance, and high shear strengths. Beside the mentioned advantages, the polyketones have another advantage since the polyketones themselves are considered biodegradable [79]. However, the drawback of such adhesives lays mainly in two factors: the limited flexibility in terms of the final formulation (emulsions characterized by solids contents below given thresholds, a function of the surfactant to PK ratio, cannot be prepared because of immediate phase separation). In addition to the fact that PK are not currently available on the market (in this respect the production costs, strongly dependent on the Pd catalyst one, do not allow the insertion of these polymers in the same category as other bulk polyolefins.

1.5 Thesis outline

The aim of this thesis is to use the proteins (Soy and *Jatropha*) in the standard polyketone-based formulations for preparing formaldehyde-free wood adhesive. In addition to this, a better understanding of the role of proteins in these emulsions is investigated.

Chapter 2 focuses on the chemical modification of the alternating polyketones by using a series of amino acids via the Paal-Knorr reaction. Two synthesis pathways, i.e. conventional and microwave pathways have been tested. The chemical reactivity of the polyketones with the amino acids and the characterization of the resulting products (polyaminoacids) as surfactant with the advantage of having the chiral properties have been studied. These novel materials open a new pathway for further possible applications.

Chapter 3 deals with the use of soy protein in polyketones-based wood adhesives. The stability and the structure of the emulsions were studied with respect to storage time at room temperature. The performance of the obtained adhesives was evaluated according the European Standard (EN-314) for wood adhesives testing.

In ***Chapter 4***, a mechanistic insight with an explanation of the role of soy proteins as co-surfactant and a thickening agent in the wood adhesives based on polyketones is given. Based on results from the systematic study on changing the ratio between the surfactant and the proteins beside the ratio between the polyketone and the protein, a statistical model, which predicts the performance (shear strength) of the emulsions after one day, is reported.

Chapter 5 deals with the extraction of the Jatropha proteins concentrate using the principle of isoelectric point precipitation. Characterization of the obtained proteins is mentioned. A study on the use of these extracted proteins in the polyketones-based wood adhesive as well as the stability and the structure of the emulsions is followed. The performance as wood adhesive was evaluated according to the European Standard (EN-314) for wood adhesive testing.

Finally, a chapter (***Chapter 6)*** about the prospects of proteins and other natural biorelated materials like chitosan in the production of polyketone based wood adhesives is presented. In addition to this, technological and scientific challenges for future research on the formulations to become techno-economically attractive are highlighted.

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Chapter 2: Reactions of aliphatic polyketones with amino acids: a flexible route to chiral polymeric surfactants

Abstract

An interesting class of polymeric surfactants was synthesized by chemical modification of alternating aliphatic polyketones with natural amino acids such as L-lysine, glycine and L-aspartic acid using the Paal-Knorr synthetic methodology. Initial studies were carried out with 2,5-hexanedione, a model compound for the alternating polyketones, and different amino acids to gain insights in the amino acids conversion rates. The reaction kinetics was shown to be a strong function of the steric bulk of the amino groups. The reaction between polyketones and amino acids was also carried out, to the best of our knowledge for the first time, under microwave irradiation, which was shown to lead to dramatic rate enhancements compared to conventional heating. The reaction products display interesting surfactant properties in aqueous solution. These have been characterized by drop tensiometry, fluorescence spectroscopy, optical active property and X-ray photoemission spectroscopy (XPS). The presence of chiral groups opens a wide number of applications for these new polymeric surfactants, for instance in chiral separation processes.

2.1 Introduction

Perfectly alternating thermosetting polyketones are available by copolymerization of carbon monoxide and unsaturated hydrocarbon monomers such as ethylene, propylene and styrene using homogeneous palladium-based catalysts [1-7]. Relevant properties of the polyketones include biodegradability, prominent photo-degradability, high wear and chemical resistance to acids, bases and solvents, stability against electrolytic corrosion and ease of functionalization [2-4,7-9].

A wide variety of synthetic methodology is available for polyketone modification. Well known products are polypyrroles, polyalcohols, polyamines, polyphenols, polythiols [4,9-11] which display interesting properties and may have potential for applications in areas like film coatings, adhesives, membranes, packaging materials, and electronic devices [1,3,4,7,10]

The Paal-Knorr condensation has proven to be a very versatile modification strategy [3,11-15] for pyrrole formation. It involves the reaction of two adjacent carbonyl groups (1,4-dicarbonyl moiety) on the polyketone backbone with a primary amine to form a pyrrole ring and water as a side product [12,16,17]. The reaction has been demonstrated for high molecular weight polyketone derivatives as well as for the low molecular weight analogs.

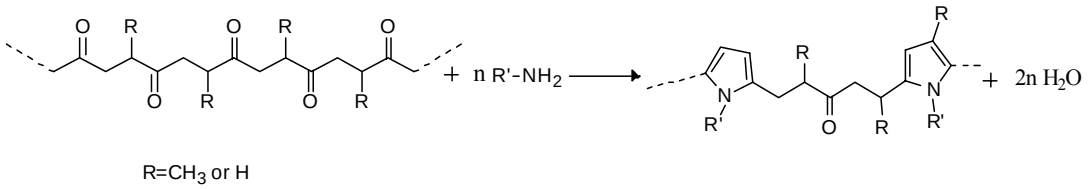

Figure 2.1: Reaction scheme for the Paal- Knorr reaction.

The resulting poly-pyrroles display good processability, high air and thermal stability [3,16]. These properties make the polypyrroles suitable for many applications, for example as building blocks for intelligent polymeric materials for sensing, information processing [3,16]. Very interesting amine containing compounds for the Paal-Knorr reaction are amino acids and derivatives thereof. This leads to products with pyrrole rings in the polymer backbone and pendent carboxylic acid functionality [11,13].

We describe here a simple, one pot synthesis of polymeric amine based surfactants by the reaction of a range of low molecular weight polyketones (M_w < 3700) with a wide range of natural amino-acids using the Paal-Knorr methodology (*Figure 2.1*). The chemistry is simple and allows for the synthesis of polypyrroles with pendant carboxylic and amino functionalities. Two different synthetic pathways were explored, a conventional method and an alternative using microwave assisted irradiation. The latter has, to the best of our knowledge, not been previously described in the literature. The effects of the type of amino acid, presence of a catalyst, molar ratio of reactants, reaction medium, temperature, and the ethylene content in the polyketone on the chemical reactivity were explored in detail. Relevant physical properties of the resulting polypyrroles were explored like surface activity (drop tensiometry), optical rotation and spectroscopic features (fluorescence spectroscopy) and the results will be reported as well. The wide range of amino acids employed in the reaction, the use of microwave irradiation as well as the characterization of chiral polymers are relevant novelties of the present work.

2.2 Experimental part

2.2.1 Materials

A number of perfectly alternating polyketones based on ethene and propylene with 0 % (PK0), 30 % (PK30) and 50 % (PK50) ethene based on the total olefin content with mass average molecular weights (M_w) of 1400, 2670 and 3632 respectively were synthesized according to a reported procedure [5]. 2,5-Hexanedione (Acros Organics, 97 %), glycine (Gly-99+ %-Sigma), L-alanine (L-Ala-≥98 %- Sigma), L-lysine (L-Lys-≥98 %-Fluka), L-aspartic acid (L-Asp-98+ %- Sigma), L-serine (L-Ser->99 %-Sigma). Triethylamine (TEA, Merk), salicylic acid (Acros), hydrochloric acid (HCl-37 %-Merk), sodium hydroxide (-> 99 %- Merck), tetrahydrofuran (THF-99+ %- Acros), dimethylsulfoxide (DMSO-99.7 %- Acros), chloroform (99.5 %- Analytical Science-Lab Scan), methanol (99.8 %- Analytical Science-Lab Scan) were all used as received. Double distilled water was used in all experiments.
3-Trimethylsilyl-1-propanesulfonic acid sodium salt (DSS-97 %-Aldrich) was used as reference for the NMR spectra. Deuterated chloroform ($CDCl_3$-99.8 atom % D- Sigma-Aldrich), deuterated tetrahydrofuran (THF-d_8-99.5 atom %-Sigma-Aldrich), deuterated DMSO (DMSO-d_6- 99.9 atom % D- Sigma-Aldrich) and deuterated water (D_2O-99.9 atom % D- Sigma-Aldrich) were used as solvents for the NMR measurements.

2.2.2 Analytical Measurements

2.2.2.1 1H-NMR. The spectra were recorded on either a Varian Mercury Plus 400 or a 500 MHz spectrometer. The assignment of the peaks was done by referring to literature data and using computer simulations using the ACD labs (11.0) software package.

2.2.2.2 Elemental analysis. C, H, and N analysis were performed using an Euro EA elemental analyzer. The data were used to calculate the conversion of the carbonyl groups and the amino acids according to the following equations:

$$X_{CO}\% = \frac{1200 \cdot z \cdot \%N}{7 \cdot x \cdot \%C - 6 \cdot y \cdot \%N} \qquad (2.1)$$

$$X_{AAc}\% = \frac{X_{CO}}{2 \cdot r} \qquad (2.2)$$

where: % N and % C are elemental analysis data, x is the number of N atoms in the amino acids, y is the number of C atoms in the amino acid, z is a constant with values 4, 3.7, 3.5 in case of PK0, PK30 and PK50 respectively, and r is the initial molar ratio between the amino acid and carbonyl groups.

2.2.2.3 FT-IR spectra. A Perkin-Elmer Spectrum 2000 was used to record the infrared spectra of the reaction products. The products were placed on a diamond plate and 30 scans were recorded for each sample with a resolution of 4 cm^{-1} over a range of 4000-500 cm^{-1}.

2.2.2.4 Surface tension measurements. The measurements were performed on a drop volume tensiometer (Lauda-TVT1) equipped with a Lauda RM6 temperature controller. The measurements were performed at a controlled temperature of 25 ± 0.1 ºC. The inner radius of the capillary was 1.055 mm and the volume of the syringe was 500 μL. The sensitivity (instrumental error) for the interfacial tension data is 0.1 mN/m [18]. The surface tension value for double distilled water (71.98 mN/m) was measured and taken as a standard before the measurements were performed [19].

2.2.2.5 Fluorescence spectroscopy. The fluorescence measurements were performed on a Perkin Elmer L550B Luminescence Spectrometer. The samples were dissolved in double-distilled water, the spectra were recorded between 365-650 nm and an excitation wavelength of 350 nm. The slit width of the emissions was kept at 3 nm step size.

2.2.2.6 Specific optical rotation. The samples were measured in water solutions using a Polartronic MH8 (Schmidt + Haensch GmbH & Co) at the wavelength of the yellow sodium line (589.44 nm). The specific rotation was calculated according to the following equation (2.3):

$$[\alpha] = \frac{\alpha}{c.(a/\ell)} \qquad (2.3)$$

where: *[a]:* specific rotation (º), *a* is the measured rotation in angular degree (º), *c* the concentration of the sample in g/100 cm^3 solution, *a* constant equal to $1*10^{-3}$ (cm^4/g), and l is the length of the sample holder tube in mm [20].

2.2.2.7 X-ray Photoemission spectroscopy (XPS). The measurements were performed using a SSX-100 (Surface Science Instruments) photoemission spectrometer equipped with a monochromatic Al Kα X-ray source ($h\nu$= 1486.6 eV). The polymer films were prepared by drop casting on gold films supported on mica. The base pressure in the spectrometer during the measurements was 10^{-10} mbar, the photoelectron take off angle was 37°. The energy resolution was set to 1.3 eV to minimize measuring time. XPS binding energies were referenced to the gold signal at 84.0 eV. Spectral analysis included a linear background subtraction and a peak deconvolution employing Gaussian and Lorentzian functions in a least-square curve-fitting program (WinSpec) developed at the LISE, University of Namur, Belgium.

2.2.3 Model component reactions of 2,5-hexanedione with various amino acids: Determination of the kinetics by ^{1}H-NMR

The reactions between 2,5-hexanedione and a variety of amino acids (L-Lys, Gly, L-Asp, L-Ala and L-Ser), were performed in an 5 mm diameter NMR tube. In a typical experiment 2,5-hexanedione (15.46 mg, 0.135 mmol) was dissolved in the deuterated DMSO (0.2 ml). The amino acid (0.135 mmol) was dissolved in deuterated water (0.5 ml) and added to the diketone solution. The solutions were stirred for about one minute at room temperature and then transferred into the NMR tube and placed in the NMR machine. Spectra were recorded at regular intervals (25 min) at a temperature of 60 ± 1 °C. The total reaction time was 14 h. For L-Lys, the reactions were performed at four temperatures (50, 60, 70, 80 °C).

2.2.4 Model component reactions: Preparative reaction for 2,5-hexanedione with L-Lys

2,5-Hexanedione (4.38 mmol, 0.515 g) dissolved in THF (3 ml) and L-lysine (4.38 mmol, 0.64 g) dissolved in water (7 ml) were mixed into a glass reactor (50 ml) equipped with a magnetic stirrer at 60 ºC. After 8 h, the solvents were removed by evaporation under vacuum (100 mbar). The resulting solid was characterized by ^{1}H-NMR.

2.2.5 Preparative reactions of polyketones with amino acids using conventional heating

The preparative reactions with conventional heating were carried out using a published procedure [11] with slight modifications. The reactions were performed in a glass reactor (50 ml) equipped with a magnetic stirrer, a reflux condenser, and an oil bath for heating. First, the polyketone (3.0 g, 0.023 mol di-carbonyl units in the PK) was dissolved in methanol (30 g) and stirred at room temperature until full dissolution of the polymer. The amino acid (intakes between 0.011 and 0.02 mol) and the catalyst (TEA) (3 g, 0.029 mol) [11] were subsequently added to the mixture. The stirring speed was kept constant at 500 rpm. The reactions were carried out at reflux temperature for 10 h. During reaction, the color of the reaction mixture changed from slightly yellowish to dark brown or dark green (depending on the kind of amino acid used). After the pre-determined reaction time the solvent was removed under vacuum (100 mbar) at 40 ºC. The solid product was dried at 40 ºC in a vacuum oven (100 mbar) for 12 h. The products were further purified by dispersing the samples in water at a pH of about 2 (obtained by using 1.0 M HCl for gross and 0.1 M HCl for fine adjustment) to remove the un-reacted amino acid and the catalyst. The desired product precipitated as a solid, is separated by centrifugation and further dried in a vacuum oven (100 mbar) at 40 ºC until constant weight. The purified products were characterized by ^{1}H-NMR, FT-IR and elemental analysis. The elemental analysis data were used to calculate the amino-acid conversions (equations 2.1 and 2.2). The effect of the pH during the neutralization step was studied by using different pH levels by adding drops of sodium hydroxide solutions at several given concentrations (0.01 M, 0.1 M for fine adjustment and 1.0 M for gross adjustment).
PK-Asp (PK30, 1:0.47): Elemental analysis: 61.7 % C, 2.88 % N. FT-IR: from pyrrole rings ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2,* and from reacted CH ~ δ 5.0 ppm (c) *Figure 2.2*.
PK-Gly (PK30, 1:0.47): Elemental analysis: 64.60 % C, 3.84 % N. FT-IR: ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2,* and reacted CH_2 ~ δ 4.6 ppm (c) *Figure 2.2*.
PK-Lys (PK30, 1:0.47): Elemental analysis: 59.04 % C, 5.97 % N. FT-IR: ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2,* and from reacted CH_2 ~ δ 3.6 ppm (c) *Figure 2.2*.

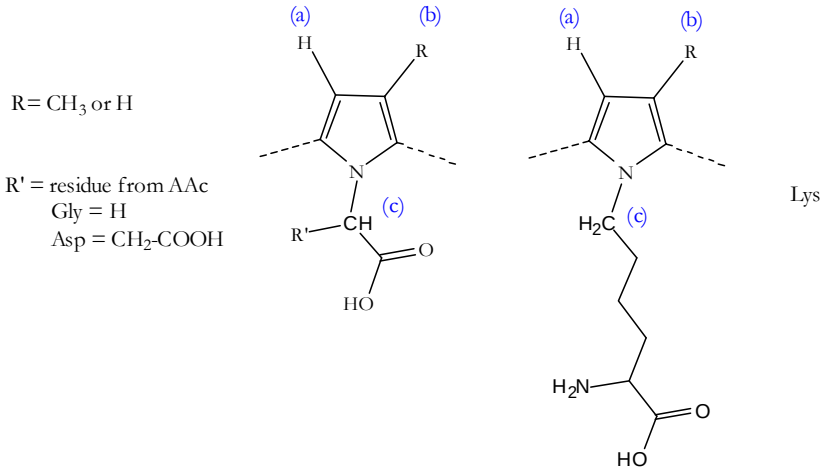

Figure 2.2: ^{1}H-NMR assignments for typical PK-AAc products

2.2.6 Preparative reactions of polyketone and amino acids using microwave heating

The reactions were performed in a glass flask (100 ml) equipped with a magnetic stirrer and a reflux condenser. First, the polyketone (3.0 g, 0.023 mol di-carbonyl units in the PK) was dissolved in methanol (30 g) and stirred. Subsequently, the amino acid (0.011 mol) and the catalyst (TEA) (3 g, 0.029 mol) were added to the mixture and transferred to the microwave apparatus. The reactions were carried out with stirring and at different reaction times (20, 40 and 60 min.) The microwave power was kept at 250 W and the temperature was set at 60 ºC. During reaction, the color of the reaction mixture changed from slightly yellowish to dark brown or dark orange for reactions with Lys and Gly and to deep purple for Asp. After the pre-determined reaction time, microwave heating was ceased. The solvent was removed using rotary evaporation under vacuum (100 mbar) at 40 ºC. The product was dried at 40 ºC and 100 mbar in a vacuum oven for 12 h. The products were purified using the procedure as described above for preparative reactions with conventional heating. Neutralization effects were studied by changing the pH of the solution by adding drops of sodium hydroxide solutions at several given concentrations. (0.01 M, 0.1 M for fine adjustment and 1.0 M for gross adjustment).
The products were characterized using elemental analysis, ^{1}H-NMR and FTIR analysis.
PK-Asp (PK30, 1:0.47): Elemental analysis: 62.72 % C, 3.21 % N. FT-IR: from pyrrole rings ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2*, and from reacted CH ~ δ 5.0 ppm (c) *Figure 2.2*.
PK-Gly (PK30, 1:0.47): Elemental analysis: 65.17 % C, 4.08 % N. FT-IR: ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2*, and from reacted CH_2 ~ δ 4.2 ppm (c) *Figure 2.2*.
PK-Lys (PK30, 1:0.47): Elemental analysis: 64.36 % C, 6.20 % N. FT-IR: ~ 3350 cm^{-1} for CH stretching and ~ 1580 cm^{-1} for ring stretching. ^{1}H-NMR: around δ 5.71 ppm and around δ 2 ppm from (a) and (b) *Figure 2.2*, and from reacted CH_2 ~ δ 3.6 ppm (c) *Figure 2.2*.

2.3 Results and Discussion

In the first stage of the investigations, model reactions were performed with 2,5-hexanedione as a model component for a polyketone and various amino acids (L-Lys, Gly, L-Asp, L-Ala, L-Ser).The employed experimental conditions are the same of those used in a previous study [3] for the reaction of the diketone with 1,2-diaminopropane. These experiments provided information about the rate of reactions as a function of process conditions and the type of amino acids. This information was applied for the synthesis of polypyrroles in a preparative scale using representative polyketones with a range of ethene/propylene ratio's (0-50 %) and molecular weights ($1400 < M_w < 3630$).

2.3.1 Model reactions between 2,5-hexanedione and various amino acids in NMR tubes

The reactions between 2,5-hexanedione and a number of amino acids were carried in NMR tubes at a range of process conditions (see experimental section). Spectra were recorded every 25 minutes for a total reaction time of 14 h. TEA was used as a catalyst in the reactions with Gly, L-Asp, L-Ala and L-Ser and DSS was used as a reference for the NMR spectra. The diketone to amino acid molar ratio was set to 1 for all experiments.

The reactions are monitored by probing the intensity of the peaks arising from the H atoms on the pyrrole rings around δ 6 ppm [3,21-25].
L-Lys is a special case as it has two amine functionalities, one attached to the chiral centre (a secondary C-atom) and one at the end of the side chain (attached to a primary carbon). Based on the known strong sensitivity of the reaction for steric hindrance [3,26], it can be anticipated that the amino group in position □ to the chiral center would be more reactive. This could not be established unequivocally with the NMR experiments, though was confirmed by preparative reactions (*vide infra*).
The conversion of L-Lys versus time was determined from the ratio of the peaks around δ 4.0 ppm (CH_2 from the reacted Lys) and around δ 3.2 ppm (CH_2 from the un-reacted Lys).
It is worth mentioning that the rest of the amino acids (Gly, L-Asp, L-Ala and L-Ser) did not show any relevant conversion under these experimental conditions even in the presence of a catalyst in the form of TEA [11]. This might be related to the sensitivity of the reaction to steric hindrance, which is considered as one of the limitations of the Paal-Knorr reaction [3,4,26]. On the basis of this reasoning, it is predicted that L-Lys is more reactive than the other amino acids also when using polyketones.
For the reaction with lysine, the influence of temperature on the conversion was investigated. The results are shown in *Figure 2.3*.

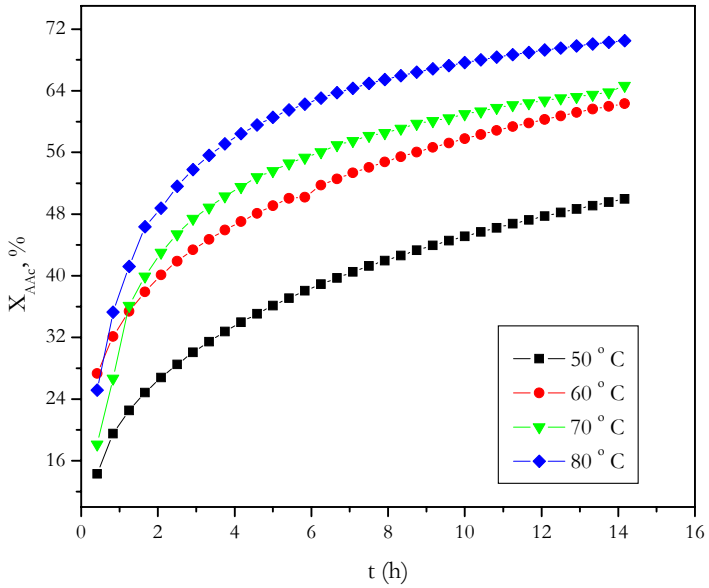

Figure 2.3: Effect of temperature and reaction time on the conversion of L-Lys in the model reactions with 2,5-hexanedione in DMSO/water solvents.

The conversions increase with temperature and time (*Figure 2.3*) reaching a maximum amino acid conversion of 71 % in 14 h at 80 ºC.
It was aimed to determine the kinetics of the reactions in detail and to obtain kinetic expressions. Unfortunately overlap of the product peaks with those of the original diketone did not allow an accurate determination of the diketone conversions as a function of time. The experimental data did not fit any simple kinetic model (first and second order in both reactants) as found for very similar systems [to be reported 27]. This suggests a complex nature of the reaction mechanism as compared with the one already proposed for simple amines [3].

2.3.2 Preparative reactions: reaction between the model component 2,5-hexanedione and lysine

In order to get further insight into the chemo-selectivity of the reaction between L-Lys and the polyketone, we performed a reaction between L-Lys and 2,5-hexanedione. This reaction was carried out on preparative scale and the crude reaction product was characterized by ^{1}H-NMR (*Figure 2.4*). Clearly, the conversion is not yet 100 % and both reacted and unreacted components are present.

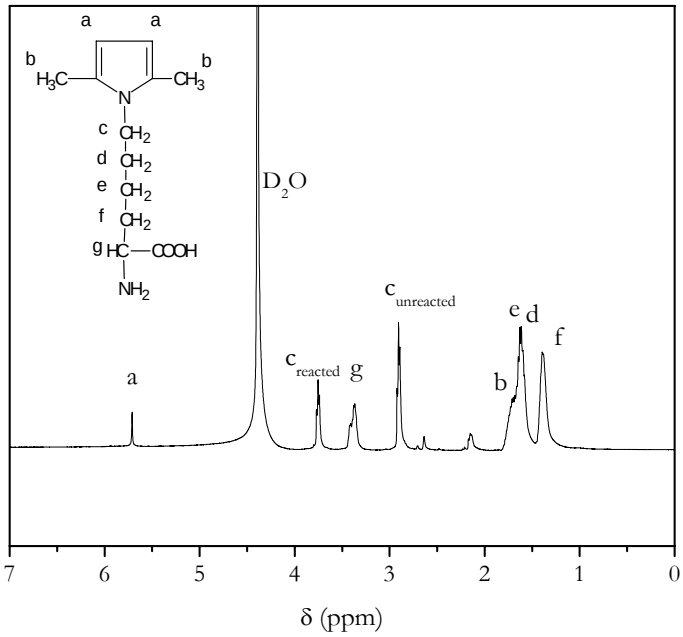

Figure 2.4: ^{1}H-NMR spectrum for the crude reaction product between 2,5-hexanedione and L-Lys in D_2O at 60 ºC.

The peak around δ 5.7 ppm arises from the protons directly attached to the pyrrole ring, the one at around δ 3.6-4.0 ppm from the CH_2 attached to the pyrrole ring while few peaks attributed to unreacted L-Lys appear at δ 3.2 ppm. The ^{1}H-NMR data and particularly the shifts of the absorption of the protons attached to the carbon indicate that the reaction takes place mainly with the amino group present in the amino acid residue (*Figure 2.4*). This reactivity pattern is not surprising when taking into account that the Paal-Knorr reaction is very sensitive to steric effects [3].

2.3.3 Preparative reactions of polyketones with various amino acids

2.3.3.1 Conventional heating

The preparative reactions between the different amino acids and polyketones using conventional heating (60 °C) were performed in methanol and TEA as a catalyst [11] (*Figure 2.5*). Polyketones with different ethylene content were applied and the molar ratio between the polyketone (30 and 50 % ethylene) and the amino acid was varied between 1:0.47 and 1:0.86.

During reaction the color of the reaction mixture changed from slightly yellowish to dark brown or dark green (depending on the type of amino acid used). After reaction, the products (*Figure 2.5*) were isolated and further purified by dispersing the samples in water at a pH of about 2 to remove the un-reacted amino acid and the catalyst. The final products were collected and dried under vacuum (100 mbar) at 40 ºC until constant weight (see experimental part). The products were characterized with FT-IR, ^{1}H-NMR and elemental analysis. FT-IR show clear vibrations of the pyrrole ring are present at around 3077-3003 cm^{-1} (stretching bands of C-H groups) and around 1500-1680 (ring stretching) [22,28]. The formation of the pyrrole product was also confirmed by using ^{1}H-NMR where the peak around δ 6.0 ppm arises from the protons directly attached to the pyrrole ring, and the one at around δ 3.6-4.0 ppm arises from the CH_2 attached to the pyrrole ring. The conversion of the amino acids was calculated using elemental analysis data of the product.

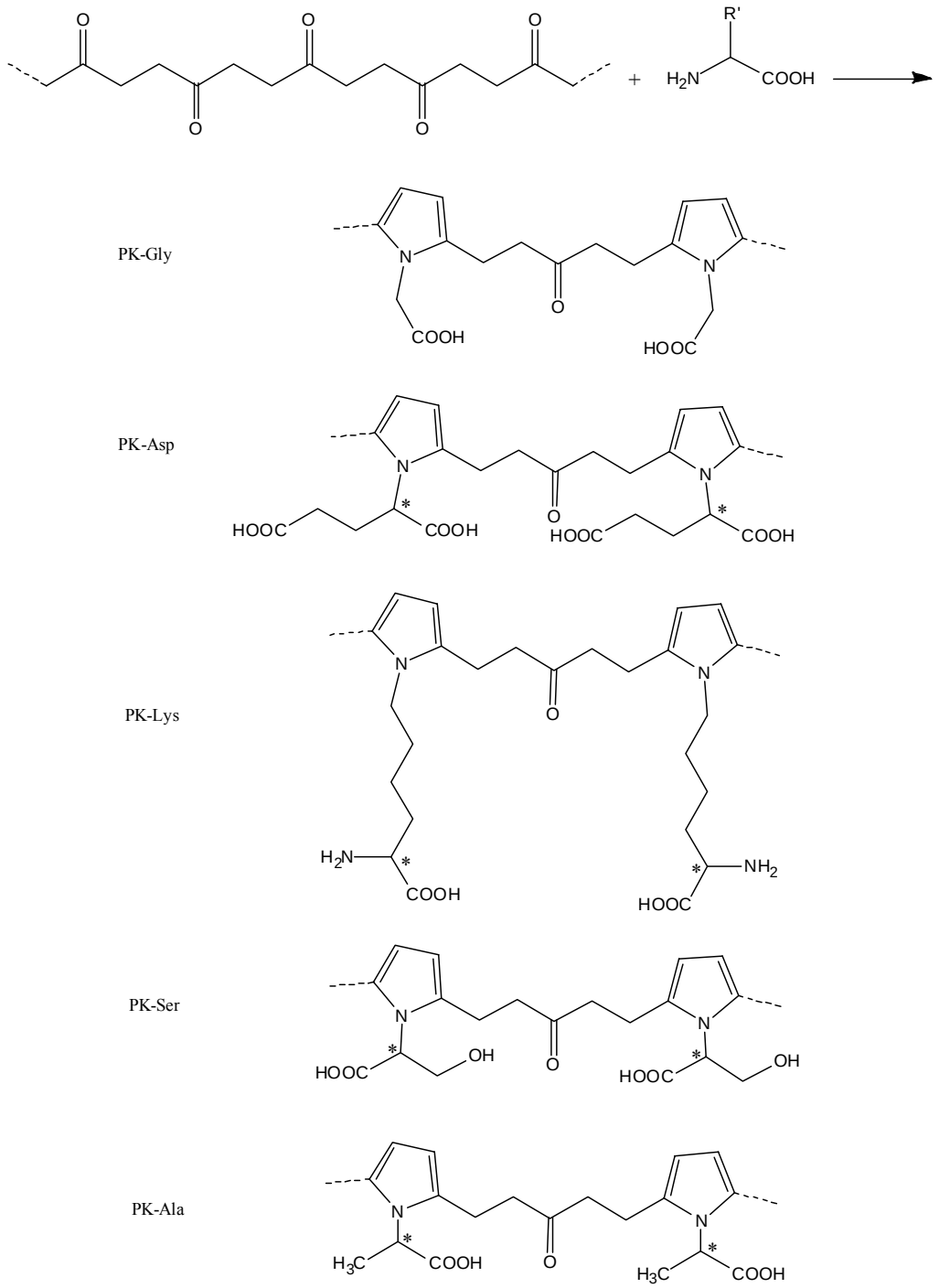

Figure 2.5: The Paal-Knorr reaction between polyketones and amino acids (PK with 100 % ethylene is drawn for simplicity), * indicates a chiral center.

The carbonyl conversion (X_{CO}) was between 35 and 79 % (Table 2.1), the latter value being close to the maximum attainable value (80 %). Quantitative carbonyl conversion is not possible on the basis of the intakes of both substrates, the reaction stoichiometry but also due to the fact that isolated carbonyl units between pyrrole rings will be formed (*Figure 2.5)* that cannot react further to form pyrroles [3].
The conversions at various intakes for Gly and L-Lys are similar within the experimental error, though Gly conversion was somewhat higher when using the PK-30 type and a

PK:AAC ratio of 1:0.86. L-Asp is considerably less active, which is not surprising taking into account the outcome of the model reactions (*vide supra*) and the differences in steric properties of the amino acids.

PK:AAc	PK-Type	AAc	X_{CO}, %	X_{AAc}, %
1:0.47	30	Gly	42	89
		L-Asp	35	75
		L-Lys	43	92
1:0.86	30	Gly	79	92
		L-Lys	66	76
1:0.47	50	Gly	48	100
		L-Asp	43	92
		L-Lys	47	100

Table 2.1: Conversion of the AAc and carbonyl groups (TEA as a catalyst, conventional heating, 60 ºC, 10 h.

The overall steric hindrance (not only the one on the reactive amino group) plays here a role as further confirmed by the use of PK50 (as opposed to PK30), i.e. of a polyketone richer in ethylene. In this case, at equal initial molar ratio of 1:0.47, the AAc conversion is almost quantitative in all cases, L-Lys and Gly still performing slightly better than L-Asp.

2.3.3.2 Reactions of polyketone and amino acids using microwave heating

A number of reactions between amino acids and polyketones were performed using microwave irradiation in methanol and using TEA as a catalyst [11]. The initial molar ratio PK:AAc was initially set at 1:0.47 and the reaction times were 20, 40 and 60 min. During reaction the color of the reaction mixture changed from slightly yellowish to dark brown or orange or deep purple (depending on the kind of amino acid used). After reaction, the products were isolated and further purified by dispersing the samples in water at a pH of about 2 to remove the un-reacted amino acid and the catalyst, the final product was collected and dried under vacuum (100 mbar) at 40 ºC until constant weight (experimental part). The products were characterized with FT-IR, ^{1}H-NMR and elemental analyses. FT-IR and ^{1}H-NMR features were as for the products obtained with conventional heating.
The results (Table 2.2) show that the conversion increases with the reaction time. Furthermore, the final conversions (i.e. after 60 minutes) are in agreement with those for conventional heating (Table 2.1), though the rate to achieve these conversions is much faster with micro-wave heating (60 minutes vs. 10 h).

PK-Type	AAc	Time (min)	X_{CO}, %	X_{AAc}, %
30	Gly	20	24	52
		40	31	66
		60	44	95
30	L-Asp	20	21	44
		40	34	71
		60	39	84
30	L-Lys	20	36	76
		40	38	81
		60	41	86

Table 2.2: Conversion of the AAc and carbonyl groups on the PK30 in solution using TEA as a catalyst, microwave assisted synthesis path at 60 ºC and 1:0.47 ratio between PK:AAc.

The reason for such higher reaction rates is likely that microwave radiation passes through the walls of the reactor and heats only the reactant and the solvent but not the reaction vessel as in the conventional heating pathway [29-32].

2.3.3.3 Further insights in reactivity and product formation

In the case of L-Lys, the reaction product consists of a polypyrrole backbone with a side chain with both an amine and carboxylic acid group. It is well possible that the pendent amine group reacts with a second polyketone fragment to form a cross-link between the two polymer backbones. This cross-link may either lead to the formation of a pyrrole ring or an imine group, see *Figure 2.6* for details.

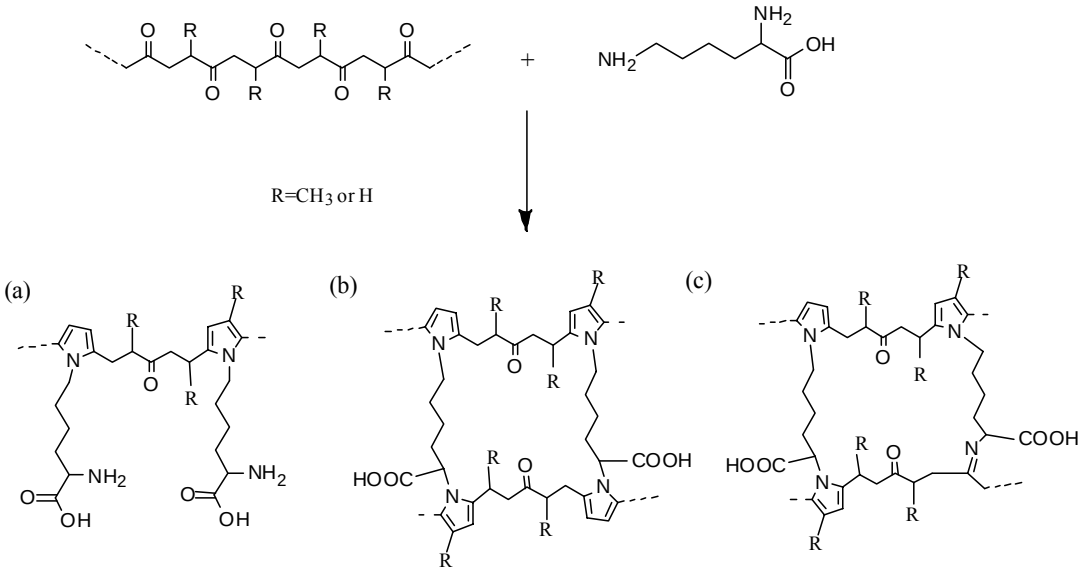

Figure 2.6: Possible reaction products of PK-Lys. (a) no cross-linking; (b) cross-linking through pyrrole formation; (c) cross-linking through imine formation.

To determine the occurrence of cross-linking reactions, L-Lys was reacted with three different types of polyketones (PK0, PK30, PK50) at 60 ºC using PK:AAc molar ratios of 1:0.2 and 1:0.47 and with either conventional (10 h) or microwave heating (1 h). The same purification method of the products was used as mentioned above. Elemental

analysis data was used to calculate the conversion of the carbonyl groups (Table 2.3) and X-ray emission spectroscopy (XPS) was used to give a quantitative elemental composition of the elements present at the surface of films prepared from the reaction products.

PK type	Synthesis type	PK:Lys	Time (h)	X_{CO}, %
PK0	Microwave	1:0.2	1	19
	Microwave	1:0.47	1	45
	Conventional	1:0.2	10	21
	Conventional	1:0.47	10	43
PK30	Microwave	1:0.2	1	26
	Microwave	1:0.47	1	41
	Conventional	1:0.2	10	23
	Conventional	1:0.47	10	43
PK50	Microwave	1:0.2	1	40
	Microwave	1:0.47	1	57
	Conventional	1:0.2	10	31
	Conventional	1:0.47	10	48

Table 2.3: Conversion of the reaction products of reaction of Lys and PK0, PK30, and PK50 with TEA as a catalyst using conventional and microwave assisted synthesis in solution at 60 ºC.

The conversion of carbonyl is in agreement with our data mentioned in the previous sections since it increases with the ethene content in the polyketone. This is due to the less steric hindrance of the carbonyl groups on the backbone in the case of the polyketone with the higher ethene content. Similar conversions of carbonyl groups were obtained by using the same kind of PK in the experiments from both the conventional and the microwave synthetic pathways (although the latter required only 1 hr reaction as compared to the 10 h of the conventional synthetic pathway). In addition, the conversion of carbonyl increased by increasing the PK:Lys ratio from 1:0.2 to 1:0.47 as expected from simple kinetics considerations. More important, we performed a surface analysis of these polymeric materials for the presence of cross-linking bridges (*Figure 2.6*). XPS analysis suggested a presence of nitrogen in the samples in many forms (*Figure 2.7*): as free amino groups at ~399.4 *eV*, as pyrrole rings at ~400.3 *eV*, as imine (Schiff base) at ~401.4 *eV*, and as second kind pyrrole ring (near the COOH groups) at ~402.7 *eV*.

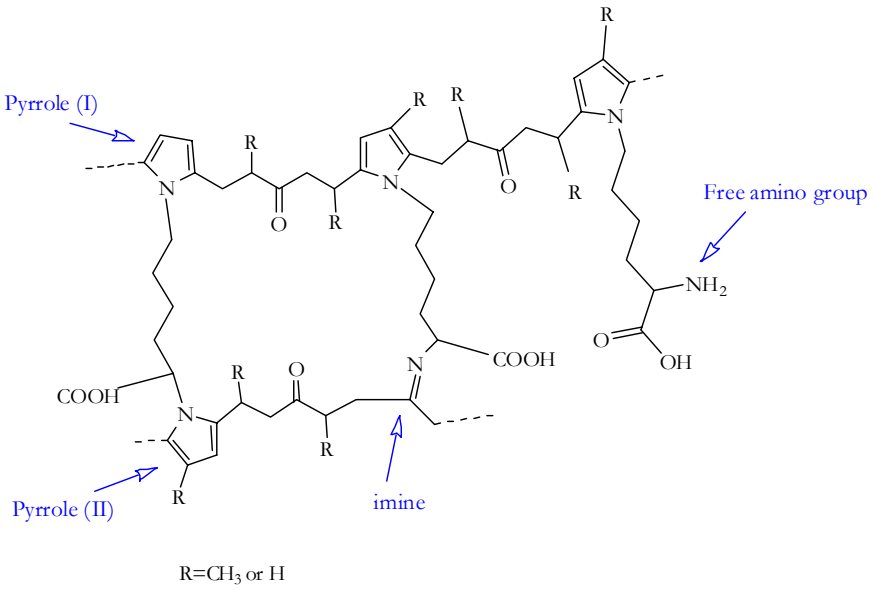

Figure 2.7: Product of PK-Lys in terms of functional groups.

The signals of the different nitrogen types present on the surface agreed with what was reported in the literature [33-35]. The XPS results clearly show (Table 2.4) that the higher the ethylene content in the polyketone (thus ideally proceeding from PK0 to PK30 and PK50) the higher is the reactivity towards the amino acid. This is clear from the disappearance of the amino group signal from the products of PK30 and PK50 and the appearance of pyrrole and double pyrrole signals instead (Table 2.4), thus clearly indicating that in such conditions also the second amino group, attached to the chiral centre, reacts with carbonyl groups along the polymer backbone.

PK type	Synthesis type	PK:Lys	Time (h)	NH_2 %	Pyrrole (I) %	C=N Imine %	Pyrrole (II) %
0	Microwave	1:0.47	1	15	43	28	14
	Conventional	1:0.47	10	12	40	31	17
30	Microwave	1:0.47	1	17	26	26	31
	Conventional	1:0.47	10	**0**	21	36	43
50	Microwave	1:0.47	1	16	20	27	37
	Conventional	1:0.47	10	**0**	58	22	20

Table 2.4: XPS analysis presenting the percentages of different types of nitrogen present on the surface of reaction products of Lys with PK0, PK30 and PK50, using the two synthetic pathways.

The quantitative percentages of the nitrogen (in its different forms) present in the system indicate that the microwave synthetic pathway results indeed in a more prominent presence of cross-linking bridges in terms of pyrrole (pyrrole (I)), double pyrrole (pyrrole (II)) and imine groups as a total (*Figure 2.7* and Table 2.4). It must be stressed here that, being XPS a surface analytical technique, the reported percentages are strictly valid (from a purely quantitative point of view) for the surface of the polymeric film. The corresponding values for the bulk of the film could be different from the one reported even if the general trends (i.e. the presence of nitrogen peaks testifying the relative reactivity of the two amino groups) can be reasonably assumed to be the same.

Nevertheless, it is worth mentioning that the solubility of the products in water tends to be less from the microwave synthesis path way compared to the conventional one, this might be also related to the formation of the second pyrrole and imine groups (thus of cross-linking points).
Further confirmation for the presence of cross-linking during the reaction comes from model reactions between 2,5-hexanedione and L-Lys using microwave heating (with ratios of DK:Lys of 1:0.2 and 1:0.47). The products were analyzed using LC-MS. Indeed, a component with mass corresponding to a bis-pyrrole was found at a molecular weight of *302.4* indicating that the other pendant amino group has reacted partially to form a second pyrrole ring.

2.3.4 Product Properties

The polypyrrole product with the pendant carboxylic (L-Lys, L-Asp, Gly) and amine/carboxylic acid groups (L-Lys) are expected to show surface activity and thus could act as surfactants [36]. To check this assumption, the products were dissolved in water up to a concentration of 0.3 mg/ml and their surface activity was measured as a function of the pH (*Figure 2.8*).

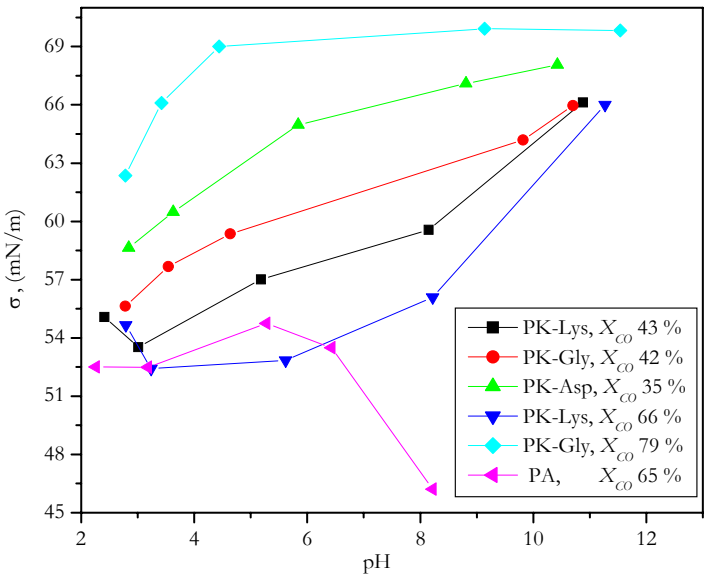

Figure 2.8: Surface tension of the products made using conventional heating as a function of the pH. X_{CO}: conversion of carbonyl group. PK-Lys: product of reaction between Lys and PK, PK-Gly: product of reaction between Gly and PK, PK-Asp: product of reaction between Asp and PK, PA: product of reaction between 1,2-DAP and PK.

The surface tensions are all lower than values for pure water and indicate that all products display a high surface activity. The surface tension is a function of the pH and increases gradually with the pH, independently of the amino acid used in the synthesis. For products with lysine groups as side chains (PK-Lys) the pH effect is less pronounced and only at a pH higher than 6, the surface tension increases. This behavior may be explained by considering the chemical composition of the pendant groups. PK-Asp and PK-Gly contain only a pendant carboxylic group. At higher pH values, these groups are gradually neutralized, thus making the polymer more soluble in water and thus decreasing its surface activity. The opposite is observed for polyketone modified with 1,2-DAP (PA in *Figure 2.8*), thus bearing free amino groups along the backbone. In this last case the

free amino groups are protonated only at low pH values and convert gradually to simple amino ones at relatively higher pH (actually precipitating from the water solution at pH > 8). The PK-Lys displays thus (*Figure 2.8*) an intermediate behavior between those of the polymers bearing only –COOH (PK-Asp or PK-Gly) or $–NH_2$ (PA) groups along the backbone. The stability, in terms of lower surface tension values, at a relatively wide range of pH is probably due to the presence of both amino as well as carboxyl acid groups on the polymer. This is not surprising if one assumes, on the basis of what is observed from the model reactions (*vide supra*), that L-Lys might react with the polyketone predominantly through its free amino group in □ position with respect to the chiral centre. This leaves both an amino and carboxyl acid groups pendant from the polymeric backbone. At relatively low pH values, amino groups are probably protonated (thus being actually ammonium groups) while carboxylic acid ones are in their original state. At relatively high pH values, exactly the opposite can be assumed. As a consequence this ensures that in a wide range of pH values a significant fraction of functional groups (either amino or carboxylic ones) is present in an ionic form. This confers to the polymeric chains the necessary hydrophilic character to explain its surface activity.
The same kind of results is also observed for the products synthesized under microwave irradiation (*Figure 2.9*).

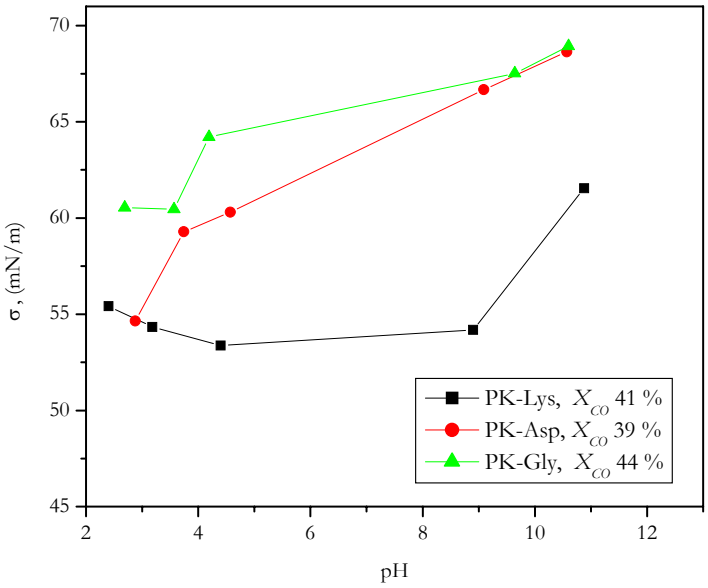

Figure 2.9: Surface tension versus pH for products made using microwave synthesis at 60 min reaction time. X_{CO}: conversion of carbonyl group. PK-Lys: product of reaction between Lys and PK, PK-Gly: product of reaction between Gly and PK, PK-Asp: product of reaction between Asp and PK.

Indeed, by comparing the products from the conventional and microwave synthetic pathways (*Figure 2.8* and *2.9*), it can be seen that for PK-Gly and PK-Asp similar results of surface activity were found. On the other hand, the product resulted from the PK-Lys modification in the microwave displays a higher surface activity than the one from the conventional reaction pathway even at higher pH. One might speculate in this case (microwave assisted reaction) that the second pendant amino group has partially reacted (*vide supra*) to produce a second pyrrole ring closure (*Figure 2.6-(b)*) or to form a Schiff

base (*Figure 2.6-(c)*) [37], thus delaying the polymer solubilization (less ionazible groups are present along the polymer backbone) in water at both high and low pH values.
The products also showed fluorescent properties, probably due to the presence of aromatic poly-pyrrole rings in the polymer backbone [38]. For PK-Lys and PK-Gly, a maximum in the fluorescence spectra was observed at around 460-475 nm for PK-Gly, and 430-450 nm for PK-Lys (molar ration of 1:0.47, conventional heating, (*Figure 2.10-(a)* and *(b)*). Higher fluorescence intensities were observed for PK-Lys, while a shift to the right and a broader distribution was found for the PK-Gly samples.
Similar results were obtained for the products using microwave heating (*Figure 2.10-(c)* and *(d)*), i.e. a maximum around 400-450 nm for PK-Gly, and 430-450 nm for PK-Lys.
The spectra are also a function of the heating method applied during the synthesis. Higher fluorescence intensities were observed for PK-Lys prepared using microwave heating. This could be due to a higher concentration of pyrrole rings or imines due to secondary cross-linking reactions.
The intensity is also a function of the pH (*Figure 2.10*). The intensity initially increases with the neutralization degree up to a maximum and then decreases again. This behavior was seen for all products (PK-Gly, PK-Lys and PK-Asp), irrespective of the heating method and molar ratios of the reactants. This effect might be due to the quenching effect due to a variety of molecular interactions such as molecular rearrangements [38].

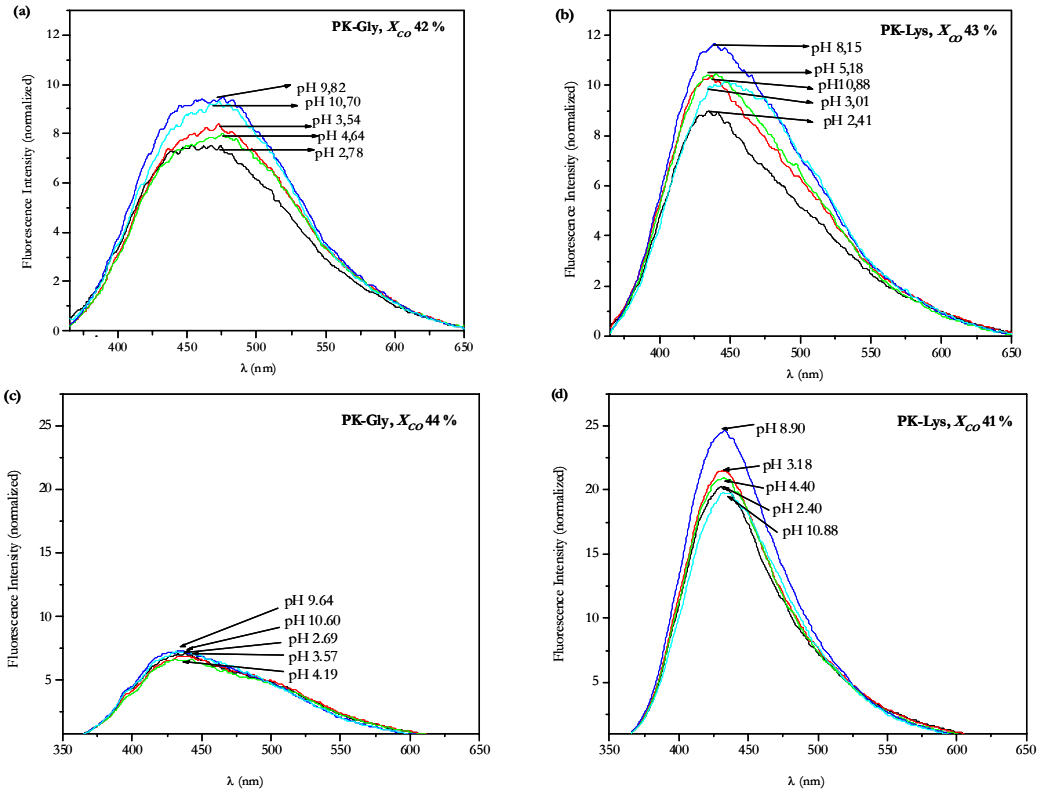

Figure 2.10: Fluorescence property of PK-Lys and PK-Gly at reaction molar ratio of 1:0.47. (a and b) conventional heating, 60 ºC, 10 hours. (c and d) microwave heating, 60 ºC, 1 hr.

Finally, the optical properties of the modified polymers were determined (Table 2.5).

Sample	$[\alpha]^{20}_{d}$, °
PK30	0
L-Lys [39]	+15
L-Asp[39]	+26
PA	+ 9 ± 0.002
PK-Lys, X_{CO} 43 %, pH=5.52	-116 ± 0.002
PK-Lys, X_{CO} 43 %, pH=8.38	-58 ± 0.002
PK-Lys, X_{CO} 43 %, pH=10.84	-81 ± 0.002
PK-Lys, X_{CO} 66 %, pH=3.17	-35 ± 0.002
PK-Lys, X_{CO} 66 %, pH=6.23	-25 ± 0.002
PK-Lys, X_{CO} 66 %, pH=11.15	-13 ± 0.002
PK-Asp, X_{CO} 35 %, pH=2.71	-29 ± 0.002
PK-Asp, X_{CO} 35 %, pH=4.6	-48 ± 0.002
PK-Asp, X_{CO} 35 %,pH=10.19	-29 ± 0.002

Table 2.5: Optical activity of the products by conventional heating.

All polymeric products of PK-Lys and PK-Asp, independently of the pH, showed optical activity.
When comparing the optical activity of PK-Lys and PK-Asp with the virgin amino acids a change in the sign of is observed. Moreover, the magnitude of the specific optical rotary power clearly decreases with the lysine content in PK-Lys (see data for 43 and 66 % carbonyl conversion). These observations suggest the possibility of a contribution of the chain conformation to the optical activity, which would then be not only dependent on the chiral centers of Lys grafted on the backbone. Finally, the optical activity is also a clear function of pH although no regular trend could be detected. This could be due to other changes in the chain conformation as function of pH (see above) or to chemical changes (i.e. neutralization) of both amino (at low pH) and acid (at high pH) groups. Although further studies are needed to fully elucidate the relationship between the polymer structure and its optical properties, one might preliminary notice how the described reactions constitute an easy synthetic pathway towards optically active polymers. The latter might find application as stationary phases in chromatographic methods for the separation of racemic mixtures. Indeed, in general the surface activity as well as the optical and fluorescence properties are important when considering future possible application of these polymers for example as surfactants, in biomedical research, household cleaning products, cosmetics, pharmaceutical aids, chiral separation of specific compounds etc. taking into consideration their biocompatibility and environmental compatibility [3,36].

2.4 Conclusions

A class of chiral polymeric surfactants was synthesized by chemical reaction between low molecular weight alternating polyketones and amino acids. The reactions were dependent on the chemical structure of the amino acid since only less steric hindered amino acids were able to provide acceptable conversion values in relatively short reaction times (10 h). The use of a microwave energy field allows easy reduction of such time up to 20 minutes, although some problems might be expected for large (industrial) scale production. The products from the microwave showed similar characterization results as the products from the conventional synthetic way. The products resulted from both synthetic pathways showed interesting properties as surfactant, which is proven by surface tension analysis. PK-Lys resulted from the microwave assisted pathway proved to be a positive exception to the general trend since it displays relatively higher surface

activity. This could be related to the more favorable conditions for the reactions, leading to formation of a second pyrrole ring (or imine) from the reaction of the pendant amino group. This hypothesis was proven by using HPLC-MS on the model compounds and XPS spectroscopy on the polymeric systems.
Furthermore, the products showed also optical active properties, which might be very interesting for future enantiomer separation for specific compounds. In addition, they displayed interesting fluorescence property in aqueous solutions. Such combination of properties by simple reaction of natural products (amino acids) with polyketones clearly indicates the flexibility of this synthetic route (especially when thinking towards industrial scale production) in providing multifunctional polymeric materials.

2.5 Abbreviations

PK:	Polyketone
PK0:	Polyketone with 0 % ethene based on the total olefin content
PK30:	Polyketone with 30 % ethene based on the total olefin content
PK50:	Polyketone with 50 % ethene based on the total olefin content
DK:	Diketone or 2,5-hexanedione
AAc:	Amino acid
Gly:	Glycine
Ala:	Alanine
Asp:	Aspartic acid
Lys:	Lysine
Ser:	Serine
TEA:	triethylamine
PK-Lys:	reaction product of PK30 and Lys
PK-Gly:	reaction product of PK30 and Gly
PK-Asp:	reaction product of PK30 and Asp
X_{CO}, %:	Conversion of carbonyl groups
X_{AAc}, %:	Conversion of amino acid groups
h:	hours
min:	minutes
σ:	the interfacial tension (mN/m)
[a]:	specific rotation (º)
a:	the rotation in angular degree (º)
c:	the concentration of the sample in g/100 cm^3 solution
l:	the length of the tube in mm
a:	constant, ($1*10^{-3}$ cm^4/g)

2.6 References

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Chapter 3: Use of soy proteins in polyketone-based wood adhesives

Abstract

This chapter describes the preparation of aqueous emulsions consisting of soy proteins and chemically modified thermosetting aliphatic polyketones in a one-pot process. Various emulsions were prepared with different total solids contents (up to 50 wt %) and different addition protocols were tested. Emulsions with 45% solids content could be prepared, a composition which results in phase separation when only polyketones are used. The stability and the structure of the prepared emulsions were studied at room temperature as a function of time using dynamic light scattering, rheology, Cryo-Scanning Electron Microscopy (Cryo-SEM) and Confocal Fluorescence Microscopy. Emulsions at 40-45 wt % total solids content with an average particle size less than 1 μm and a viscosity less than 1 Pa.s could be prepared and were shown to be stable (i.e. no macroscopic phase separation) for more than 6 months. The role of the protein in the emulsion is tentatively explained on the basis of the collected data.

The performance of the aqueous emulsions containing the soy protein and chemically modified polyketone as wood adhesive was evaluated. It is shown that the introduction of soy protein into the basic recipe of a polyketone-based adhesive, while providing extra stabilization of the corresponding emulsions (*vide supra*), does not affect in any way the wood adhesive performance of the final product. Indeed, all prepared emulsions (with protein content up to 40 wt % with respect to the unmodified polyketone) fulfill the wood adhesive requirements according to the European Standard as observed for the basic polyketone-based adhesive (i.e. without any addition of proteins). In this respect, it is clear that the main drawback of classical proteins-based wood adhesives, namely very poor water resistance, is counterbalanced by a positive influence of the polypeptidic chains on the overall performance. Indeed, confocal fluorescence pictures clearly demonstrate a better penetration of the protein/polyketone adhesive into the wood surface as compared to the one of the adhesive based on polyketones only.

The use of soy proteins in the polyketone-base formulation did not change the properties and performance as wood adhesive. In addition to that, the use of soy proteins is advantageous from economic point of view since it is low-cost filler.

3.1 Introduction

Co- and ter-polymerization of carbon monoxide and olefins such as ethylene and propylene by homogeneous palladium complexes enables the synthesis of perfectly alternating thermosetting polyketones (PK) [1,2]. Depending on the ratio between ethylene and propylene along the polymer backbone, the low molecular weight polyketones are viscous liquids at room temperature for ethylene-free polymers to waxy or low melting solids at 50 mol % ethene content [3,4]. The low molecular weight polyketones (Carilite) have successfully been applied as wood-binding adhesives [5]. High molecular weight polyketones display many interesting properties. Examples are biodegradability, photo-degradability, chemical resistance to acids, bases and solvents, and stability against electrolytic corrosion. Some applications are in automotive, electrical, containers and pipelines, fibers, film-coating, packaging material, and in optoelectronic and electronic devices [1,6].

From a chemical point of view, the reactive carbonyl groups on the backbone allow for many chemical modifications. The resulting modified products (such as polypyrroles, polyalcohols, polyamines and polyphenols) usually display novel properties with respect to the starting polyketones and are therefore suitable for many applications such as film coatings, adhesives, membranes, and electronic devices [7,8].

Among the available modification reactions, the Paal-Knorr condensation was shown to be very versatile [4,9]. In this reaction two adjacent carbonyl groups react with a primary amine to form water resistant pyrrole rings (*Figure 3.1*). Water is eliminated as a side product [4,8,10-12].

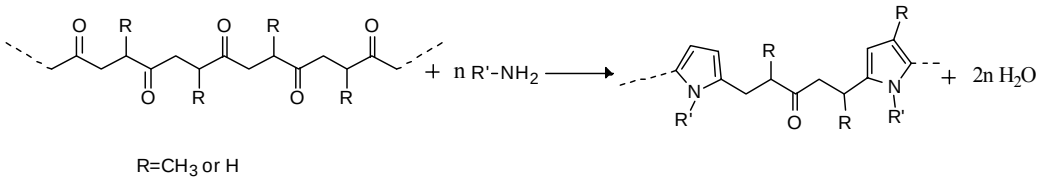

Figure 3.1: Reaction scheme for the Paal- Knorr reaction.

This reaction has been used to produce polymeric surfactants able to stabilize a dispersion of the virgin polyketone in water [4,9]. In this case (see *Figure 3.2* in the experimental part), the polyketone was reacted with a di-amine (1,2-diaminopropane, 1,2-DAP), to prepare polyamines. In this case advantage has been taken of the Paal-Knorr sensitivity to steric hindrance [4], i.e. only the less hindered amino group of 1,2-DAP (the one in position 1) is able to react with the carbonyl groups along the backbone while the second (in position 2) remains unreacted and thus pending as side chain from the polymeric backbone. The resulting polyamines exhibit double functionality, i.e. a pyrrole ring group and an amino functionality that is subsequently transformed (*Figure 3.2*) into a water-soluble cationic compound by protonation with weak acid (acetic acid in this case). The protonated polyamines acted as polymeric surfactants and were used to prepare the water-based polyketone emulsions. Such emulsions were much less viscous than the original polyketones and allowed easy deposition on substrates. These emulsions have been applied as a paste or as a spray depending on the viscosity [4,11,13]. The polyketones-based emulsions, consisting of surfactant (protonated polyamines) and polyketone at 50 wt % total solids content, displayed outstanding behavior as wood adhesives [4,11]. The products displayed advantages over commercially available wood adhesives such as urea-formaldehyde (UF) and phenol-formaldehyde (PF) resins [14-17]. According to the World Health Organization, formaldehyde based adhesives are known to be harmful to the environment and suspect carcinogenic [14,15]. The overall procedure for producing polyketone-based wood adhesives did not involve the use of

any harmful chemical components and produced only water as by-product [4]. Growing environmental awareness by general public and industry clearly expresses the necessity to develop more environmental-friendly wood adhesives [14-18]. In this respect several studies have been published concerning the use of natural proteins in glue compositions. Among all possible natural materials, soy proteins are the most popular studied [14,16,18]. They are abundantly available, inexpensive and considered as a renewable source [14,16,18]. Moreover, soy proteins can be handled with ease and can be processed at hot and cold press conditions, i.e. typical cold recommended processing pressure values are between 1.03-1.21 MPa for 15 min while the conditions for hot pressing vary depending on the wood panels thickness from 120-160 ºC at 0.981 MPa pressure for 2 min up to 230-270 ºC at 1.21 MPa for 1.5 min [14,18]. However, pure soy protein-based adhesives suffer from lower strengths, sensitivity to biological degradation and lower water resistance [14,16,18], disadvantages that are on the other hand not displayed by polyketone-based adhesives [4].
The present work investigates for the first time the use of soy proteins in polyketone based wood adhesives. Despite an obvious economical and environmental-friendly drive for replacing polyketone by these proteins in a PK-based wood adhesive, the presence of polar polypeptidic chains formulated together with the apolar PK, could help in the penetration of the adhesive into the polar wood surface (positively affecting therefore the adhesive performance). However, a dramatic change in polarity of the adhesive could also result, as observed for glues based only on proteins [14,16,18], in a dramatic decrease of the water resistance (thus negatively affecting the adhesive performance as compared to emulsions based on pure PK) [4]. Based on these considerations, it is foreseen that an optimum protein intake may be required in terms of emulsion stability and adhesive performance. The effects on the adhesive formulation, expressed in the form of protein intake, addition protocol and overall solids content were systematically studied in relation to the structure and stability of the resulting emulsions. Several measurements, such as particle size and viscosity analyses, were performed to test the stability of the final products. Cryo-SEM analysis was used to investigate the morphology while confocal fluorescence microscopy was used to determine the penetration depth of the adhesive into the bulk of the wood specimen. All formulations were tested according to the European Standard EN-314 wood adhesive test [4,11].

3.2 Experimental part

3.2.1 Materials

Polyketone with 30 mol % ethene based on the total olefin content (PK30, M_w 2670) was synthesized according to a reported procedure [2]. 1,2-Diaminopropane (1,2-DAP, 99+ %, Acros), Acetic acid (99.5 % pure, Acros), salicylic acid (reagent ACS, Acros), and Soy Protein acid Hydrolysate (Pr, Sigma-Aldrich, Mw between 5500-7000 Da measured using MALDI-TOF) were purchased and used without further purification. Commercial wood maple veneers were purchased from Sikkens Center Groningen (The Netherlands). Double distilled water was used in all experiments.

3.2.2 Emulsion preparation

The need of producing an emulsion arises from the high viscosity of the thermosetting polyketones which varies from 1.1, 2.3 up to 8.1 Pa.s at 80 ºC for polyketones with 0 mol %, 30 mol % and 50 mol % ethylene respectively based on the total olefin content. This high viscosity makes the direct deposition on the substrate difficult.

Polymeric amines were first prepared (*Figure 3.2*) by chemical modification of the polyketone (mPK30) using 1,2-DAP. Following a well-known procedure [9], the reaction was carried out in a 250 ml rounded bottom glass reactor with a reflux condenser, U-type anchor impeller and an oil bath. First the polyketone (40.0 g, 0.304 mol; calculations based on di-carbonyl units in the PK polymer) was heated in an oil bath to a temperature of 100 ºC, then 1,2-DAP was added drop-wise (18.02 g, 0.243 mol, based on initial molar ratio between 1,2-DAP and the carbonyl groups in the PK of 0.8) in the first 20 minutes of the reaction time. The stirring speed was kept constant at 500 rpm. The reactant mixture changed from a yellowish to a brown color and became a solid material upon cooling to room temperature. The prepared polymeric amines were washed several times with double distilled water, filtered and freeze dried. The final product was a light brown powder. The conversion of the carbonyl groups to pyrrole rings was determined by elemental analysis and found to be around 70 %.

These polyamines were subsequently converted to water-soluble cationic compounds by protonation with acidic acid solution in double distilled water to match a desired protonation level of 50 %.

In the emulsification step (*Figure 3.2*), a second amount of unmodified polyketone (PK30(II)), soy protein (Pr), and double distilled water were added to the protonated polyamine solution to reach the desired total solids content (50, 45, and 40 wt % respectively). The second amount of unmodified polyketone and soy protein were added according to two different addition protocols (*Figure 3.2*). In the first, the soy protein was added simultaneously with the unmodified polyketone while in the second the protein was added 1 hour before the polymeric component, i.e. immediately after the surfactant preparation by protonation. All synthesis steps were performed in a single reactor (one-pot process). The resulting emulsions were stored at room temperature in sealed high-density polyethylene (HDPE) containers and further analyzed.

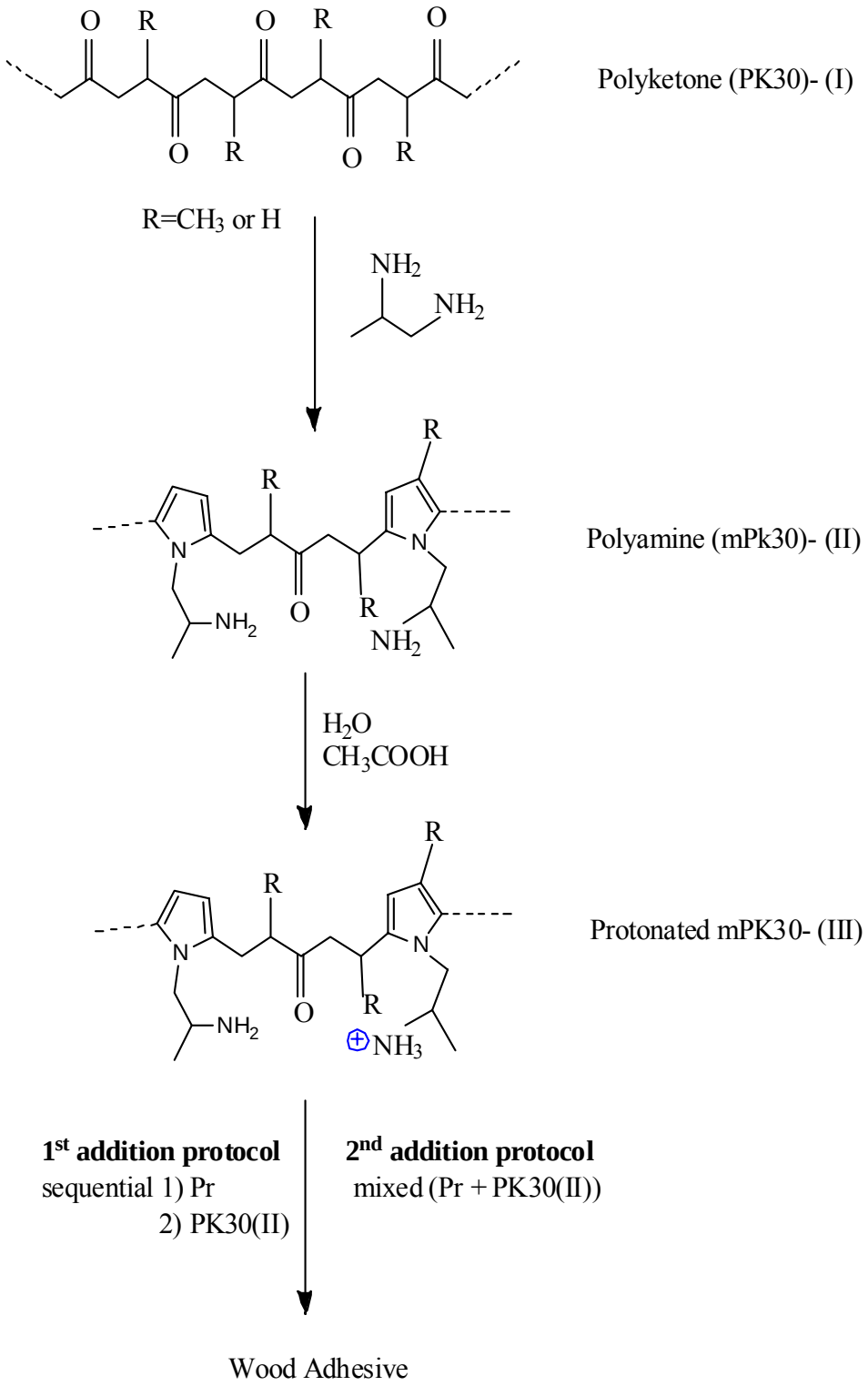

Figure 3.2: Preparation of the protein-containing aqueous emulsions.

The complete recipes for all glues are reported in Table 3.1.

Amount of mPK30, (g)	Amount of soy Pr, (g)	Amount of PK30(II), (g)	wt % soy Pr[a]
1	0	1.5[b]	0
1	0.3	1.2	20
1	0.6	0.9	40
1	0.9	0.6	60

Table 3.1: Recipes for wood glue emulsions. [a] Percentage with respect to the unmodified polyketone (PK30(II)); [b] recommended quantity PK30(II) required to achieve adhesive bond strength according European standard

Various factors are expected [4] to affect the production, stability and performance of the emulsions as wood adhesives (Table 3.2).

Factors	Value	Unit
Rotor speed	500	rpm
Emulsification time	90	min
Emulsification temperature	80	ºC
Protonation level	50	%
Polyketone (ethylene content)	30	%
Amount of added protein	20, 40, and 60	wt %[a]
Addition protocol (protein)	- Pr before (sequential) - Pr together (mixed)	
Solids content	40, 45, and 50	%
mPK30:(PK30(II)+Pr	1:1.5	wt/wt

Table 3.2: Factors influencing the preparation of wood glue emulsions. [a] Percentage with respect to the unmodified polyketone (PK30(II))

In the present work, we systematically studied the influence of the protein intake, the addition protocol and the solids content.

3.2.3 Dynamic light scattering

The average particle size of the emulsions ($\bar{d}$) was determined by using dynamic light scattering measurements (Zetasizer 5000 instrument, Malvern Instruments, U.K). The samples were diluted 1000 times in double distilled water for each analysis. The measurements were performed at a wavelength of 633 nm and a temperature of 25 ºC. Scattered light was detected at an angle of 90º. The viscosity (0.89 mPa.s) and refractive index (1.33) of water at 25ºC were used as reference for data analysis. The data were analyzed by using an autocorrelation CONTIN algorithm to obtain the intensity average hydrodynamic diameter. The average particle size is calculated from the diffusion coefficient by using the Stokes-Einstein equation [19];

$$d(H) = \frac{kT}{3\pi\eta D} \qquad (1)$$

where: *d(H)* is the hydrodynamic diameter (nm), *k* Boltzmann's constant (J/K), *T* the absolute temperature (K), η the viscosity (Pa.s) and *D* the diffusion coefficient (m^2/s).

3.2.4 Rheological analysis

The viscosity of the polyketones (η) was measured at 80 ºC by using an AR 1000 Rheometer (TA Instruments, USA) with using aluminum cone-and plate fixture of 4º

cone-angle and 20 mm diameter. The apparent viscosity of the polyketones was measured at a constant shear rate of 5 s^{-1}. The emulsions viscosity (η) was measured at 20 ºC using aluminum cone-and plate fixture of 2º cone-angle and 40 mm diameter. The apparent viscosity of the samples was measured at constant shear rate ($\dot{\gamma}$) of 5 s^{-1} for 50 wt % solids content samples and 15 s^{-1} for 45 and 40 wt % solids content samples. The samples with a 50 wt % solids content were measured at lower shear rate because of their very high viscosity, which exceeded the safety limits of the machine. The viscosity-shear rate relationship was established by measurement at different shear rates in the range from 1-10 s^{-1} and 5-60 s^{-1}.

3.2.5 Wood adhesive testing

The wood pieces of maple veneers for the adhesive test were dried at 105 ºC for 10 h to reduce the moisture content to a constant level. A given amount of salicylic acid (0.5 wt %, based on the second amount of polyketone (PK30(II)) and soy protein) was used in the emulsion as a curing catalyst.

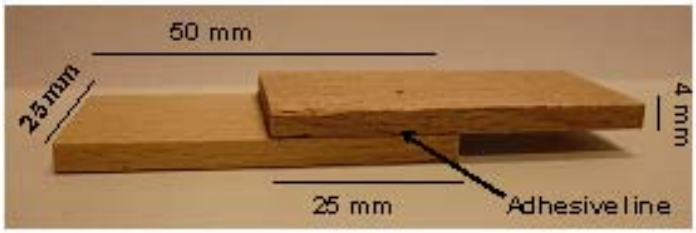

Figure 3.3: Specimen dimensions for shear test

The emulsions were applied at 150 g/m^2 single adhesive line onto one side of $(25 \times 50 \times 4)$ mm^3 maple veneer pieces. The area where the glue was exposed on every piece was (25×25) mm^2 (*Figure 3.3*). The specimens were hot-pressed for 5 minutes at 200 ºC under constant pressure of 3 MPa. Ten to thirteen replicates were tested for each experiment. In order to determine the bond quality of maple veneer adhesives according to the European Standard test EN-314, the specimens must undergo several pretreatments before the shear tests are applied. The specimens were first immersed in boiling water for 72 hours and then cooled in water for at least one hour to room temperature. The shear strength ($\sigma_{strength}$) was measured by using an Instron 4301 machine using 5 KN power sensor with a crossing speed of 2 mm/min.

3.2.6 Cryo scanning electron microscopy (Cryo-SEM)

Cryo-SEM (JEOL 6301 F) was used to examine the structure of the emulsions. The cryo unit used was Oxford CT 1500 HF Cryotransfer system. A drop of (three times) diluted emulsions was placed on a filter paper of 0.2 μm using special tissue adhesive. The samples were then placed on the cryo-specimen holder and were cryo-fixed into slush nitrogen at -210 ºC. After that the specimens were transferred in a frozen state to the cryo-unit. The samples were then sublimed for 10 minutes and sputter coated with Au/Pd at -120 ºC to have a layer of approximately 3 nm thickness. Finally the samples were imaged and SEM pictures were recorded at -120 ºC.

3.2.7 Chain length calculations

Amino acid composition analysis on soy protein was performed by Eurosequence B.V Analysis and Synthesis of Protein and DNA, Groningen, the Netherlands.

mPK30 and Pr chain length calculations were estimated based on the computer program Molecular Modeling Pro Plus, version 6.2, using the zigzag configuration and data of standard bond distances of amino acids and peptides [20].

3.2.8 Confocal Fluorescence Microscopy

The Confocal fluorescence measurements were performed using Leica SP2 (AOBS Confocal Microscope) with mercury lamp of 50 W at a magnification of (X10) and 400 Hz scan speed. The images were recorded between 501 and 597 nm. Similar samples to those for the shear test were first sliced and then scanned on the glue line.

3.3 Results and Discussion

Several emulsions were prepared by ideally replacing, in the basic polyketone emulsion recipe, different amounts of the unmodified polyketone with different amounts of soy protein (Table 3.1) up to 60 wt %. Indeed, attempts to increase the protein intake even up to 100 % (thus practically substituting all the unmodified PK30(II) in the formulation) resulted either in the formation of pastes (high viscosity) or even in complete failure during the wood adhesive test (during boiling, see experimental part). The addition of proteins was performed according to two different protocols and at different percentages of solids content, *Figure 3.2* and Table 3.2. All prepared emulsions were compared with a reference sample where no protein was added. In this preparation procedure, i.e. during the emulsification process, different amounts (20, 40 and 60 wt % with respect to the unmodified polyketone intake) of protein were added with the unmodified polyketone PK30(II). All samples were prepared by keeping the ratio between the soy protein and PK30(II) intake to the modified polyketone (mPK30) equal to 1.5**.** In this study three different solids contents were prepared for every emulsion (50, 45 and 40 wt %).
The presence of proteins has a very positive effect on the stability of the polyketone dispersion. Emulsions at 40 and 45 wt % solids content without the addition of soy protein could not be prepared and resulted in immediate phase separation. The addition of a small amount (20 wt %) of protein was sufficient to avoid any instability problem (*Figure 3.4*).

Figure 3.4: Stable and unstable emulsions. Left: sample at 40 wt % solids content and no addition of protein. Right: sample at 40 wt % solids content and 20 wt % protein with respect to the unmodified PK30(II).

Although the exact reason for this positive effect of proteins on emulsion stability is currently under investigation, one might preliminarily speculate that the protein is able to absorb at the surface of the polyketone particles, thus providing an extra stabilization (electrostatic- or steric) to the system [21], thus practically acting as co-surfactant.

The emulsion stability is one of the main requirements for application as wood adhesives. The stability was examined visually (*Figure 3.4*) and from the changes in the average particle size with respect to storage time. The obtained emulsions are stable at ambient temperature for at least two months and their average particle size increases slightly at the beginning and then levels off with time (*Figure 3.5*).

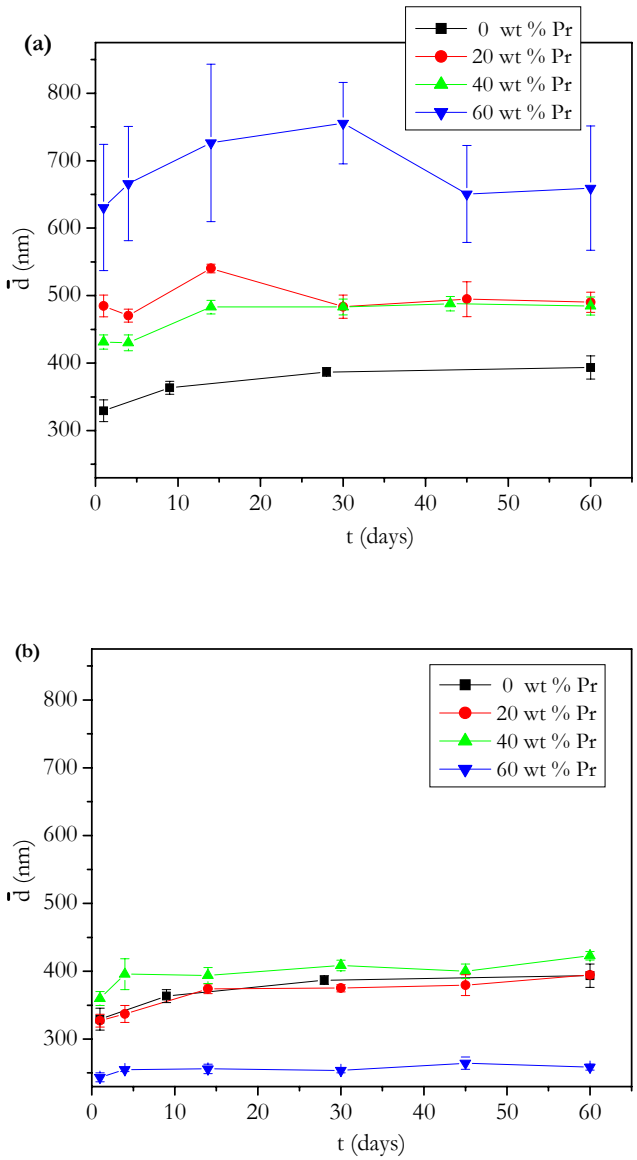

Figure 3.5: Effect of storage time and protein content on particle size of the emulsions (50 wt % total solids content). (a): protein added at the same time with unmodified PK30(II), (b): protein added before the unmodified PK30(II). (Pr refers to soy protein).

The presence of soy protein has definitely an influence on the average particle size. For both mixing protocols the addition of up to 40 wt % of soy protein results either in comparable average particle size (*Figure 3.5-(b)*) or in an increase of the latter with respect to the reference sample (0 wt % protein) (*Figure 3.5-(a)*). On the other hand, samples

containing 60 wt % protein show a totally different trend and the effect is a strong function of the mixing protocol. If the protein is added together with the unmodified polyketone (*Figure 3.5-(a)*) the resulting emulsion displays a significantly higher average particle size than the other samples. Exactly the opposite trend is observed when the protein is added before the unmodified polyketone (*Figure 3.5-(b)*). The observed discrepancy is not an artifact of the light scattering analysis as it is further confirmed by SEM (*Figure 3.6*).

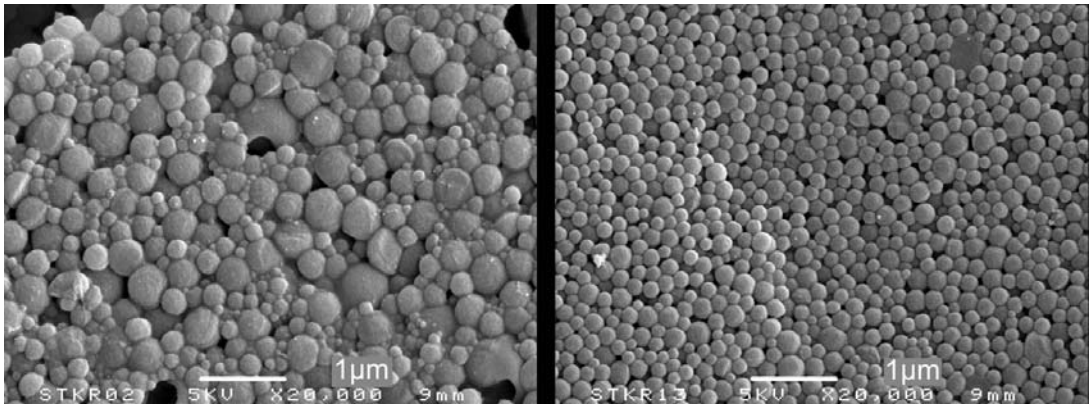

Figure 3.6: Cryo-SEM pictures of emulsions 20 wt % (left) and 60 wt % protein (right), 50 wt % solids content, protein added before the unmodified polyketone.

Indeed, the SEM pictures clearly show that the sample with 60 wt % protein has a much lower average particle size than the sample with 20 wt % protein. This result is quite general in this study as it also applies for systems with different overall solids contents (not shown here for brevity). Thus, the addition protocol is a very important variable and has a significant effect on the morphology of the resulting emulsion. This is further confirmed by comparing the two graphs in *Figure 3.5*. The addition of the protein before the unmodified polyketone always results in a lower average particle size than the case when adding the two components simultaneously.
Finally, the slight increase in the particle size with time at the start (*Figure 3.5*) can probably be attributed either to Ostwald ripening or coalescence. In this process larger droplets tend to grow at the expenses of smaller particles. This is one of the main mechanisms for emulsion destabilization [21-23]. After this first increase, the particle size remains stable in time as is further confirmed by the cryo-SEM pictures (*Figure 3.7*), which show uniform spherical shape particles with even a slightly finer dispersion after 6 months storage time.

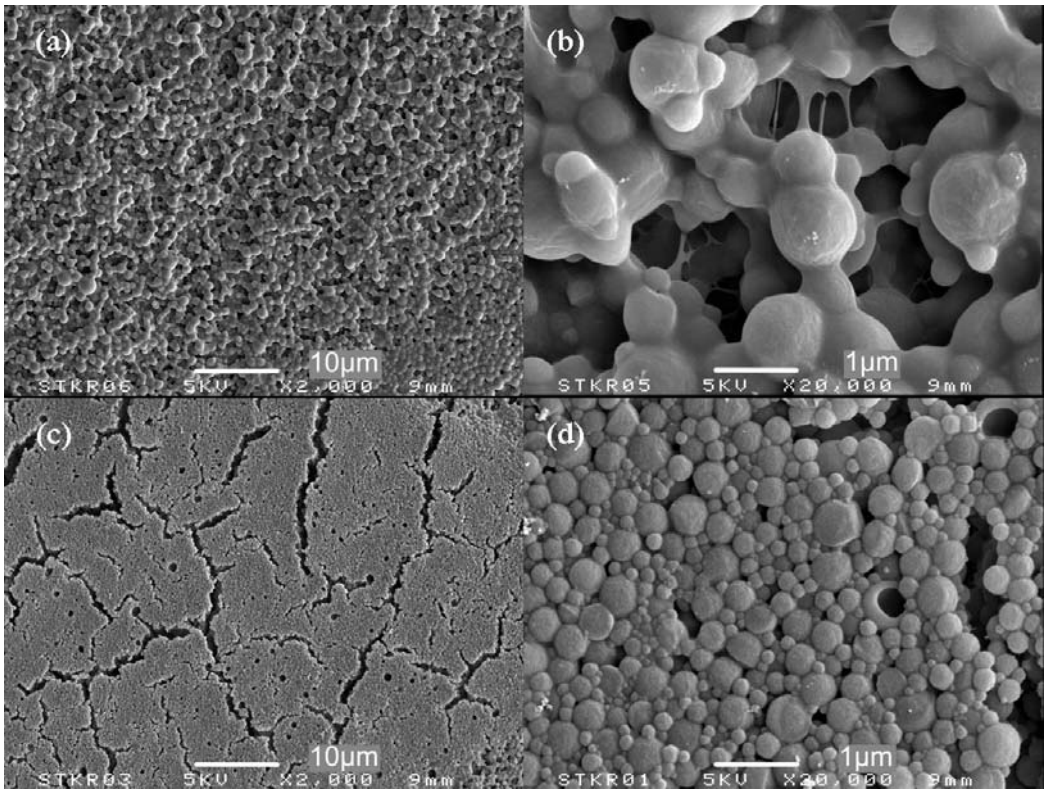

Figure 3.7: Cryo-SEM pictures of emulsions 20 wt % protein at low and high magnification. (a and b) freshly prepared samples (c and d) after 6 months' storage time.

The rheology of the emulsions was studied as a function of the protein contents, addition protocol and solids contents. Three emulsions at different solids contents were prepared for a given intake of protein. Lower viscosity was measured at lower solids contents (*Figure 3.8*) as expected due to the more diluted nature of the system.

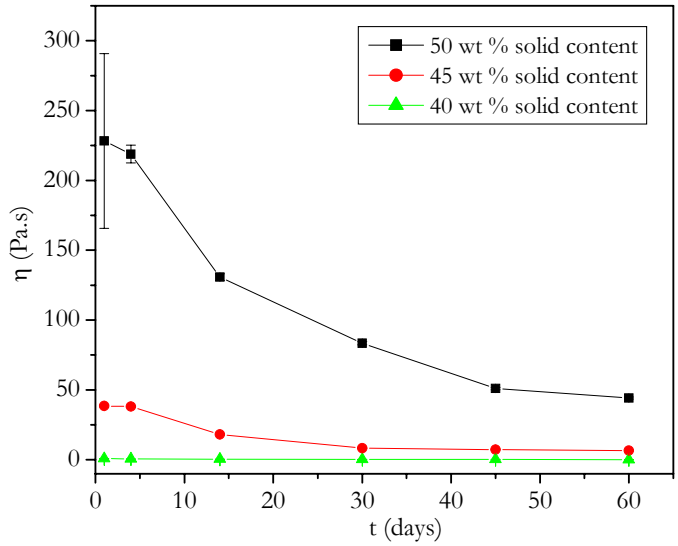

Figure 3.8: Effect of storage time on viscosity of the emulsions. 20 wt % Protein at different total solids contents. Protein added at the same time with unmodified PK30(II).

Besides the dependence on the solids content, the viscosity is also a clear function of the amount of protein present in the formulation as well as of the addition protocol (*Figure 3.9*). All samples containing 20 and 40 wt % protein display, independently of the addition protocol, a higher viscosity than the reference sample (no protein present). This confirms our initial hypothesis (*vide supra*) that the protein might act as a thickening agent, thus causing an increase of the viscosity but also an extra (steric) stabilization of the dispersion. However, such differences are a clear function of the addition protocol (compare the two graphs in *Figure 3.9*). The addition of the protein before the unmodified polyketone results in all cases in a lower viscosity compared to the case when adding the two components at the same time. Also in this case, like for the average particle size data, the sample containing 60 wt % of protein does not follow the general trend outlined above; this sample had a very high viscosity which could not be further measured at the same conditions due to exceeding the safety limits of the machine.

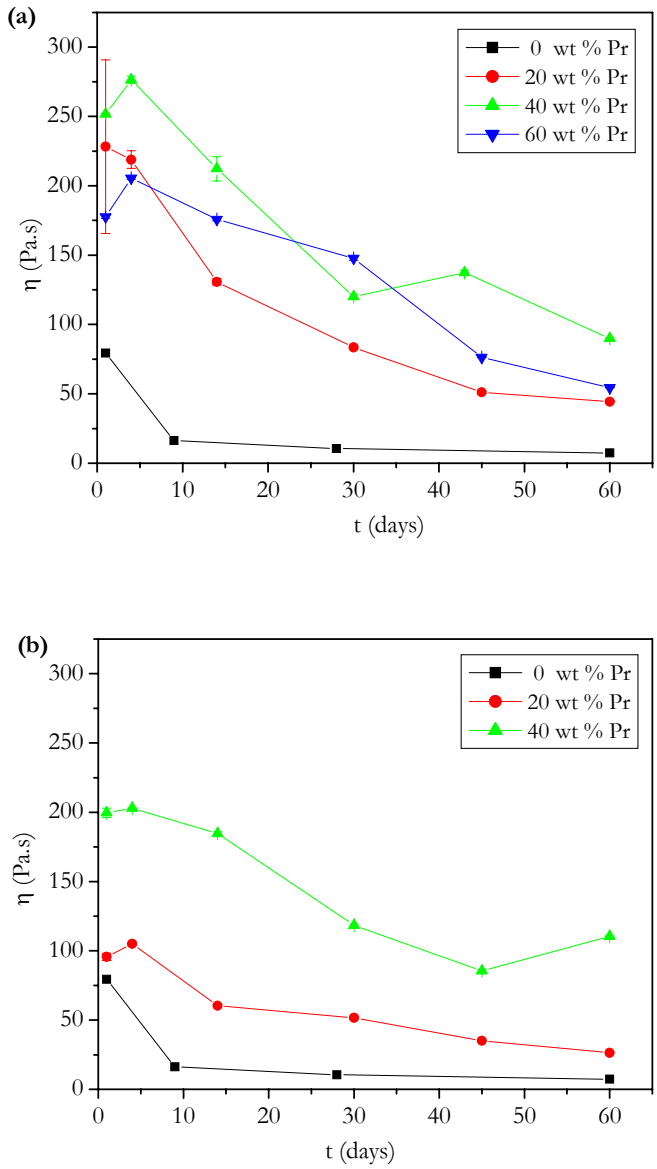

Figure 3.9: Effect of storage time on viscosity of the emulsions. (50 wt % total solids content). (a): protein added at the same time with unmodified PK30(II), (b): protein added before unmodified PK30(II).

Furthermore, it can be seen that there is a sharp decrease of the viscosity in the first four to five days. No significant decrease is noticed in the following two months storage time. The sharp decrease of the viscosity could not be related to the particle size since the latter is not changing dramatically. However, this phenomenon has been already observed in our previous work on polyketones (without proteins) based adhesives [4]. In that case we proposed that the sharp decrease could be explained in terms of the polymer rearrangement/relaxation within each particle. Also in the present case (thus with proteins in the basic formulation) the same explanation is probably valid. This is indirectly confirmed by the fact that the samples at the beginning reveal non-Newtonian behavior and change in time to Newtonian (*Figure 3.10*).

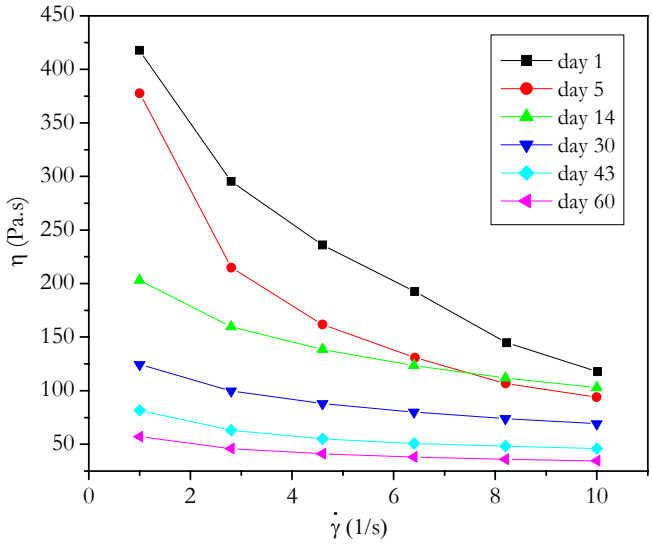

Figure 3.10: Effect of shear rate on the viscosity of the emulsions. Protein added at the same time with unmodified PK30(II), 20 wt % protein, 50 wt % total solids content.

Since shear thinning (non-Newtonian behavior) is typical of (polymeric) systems in which the chains form entanglement with each other, one might suppose that the initially stretched surfactant chains at the surface of the polyketone particles gradually and partially retract in time closer to the surface of the particles, thus providing a lower chance for the formation of entanglements and hence the transition to Newtonian behavior [4]. The main difference between the present system and the binary system without protein [4]) is that in the binary system, the effect is attributed to the surfactant (modified polyketone) only, but in the ternary system the effect is due to the polypeptide (protein) chain as well as to the surfactant (modified polyketone-mPK30). Such difference between the binary system (PK30(II) and mPK30) and the ternary one (PK30(II), mPK30 and Pr) is actually crucial since it possibly explains the role of the protein in determining the emulsion stability and rheology. A schematic representation of the hypothetical particle structures in the two situations is depicted in *Figure 3.11*.

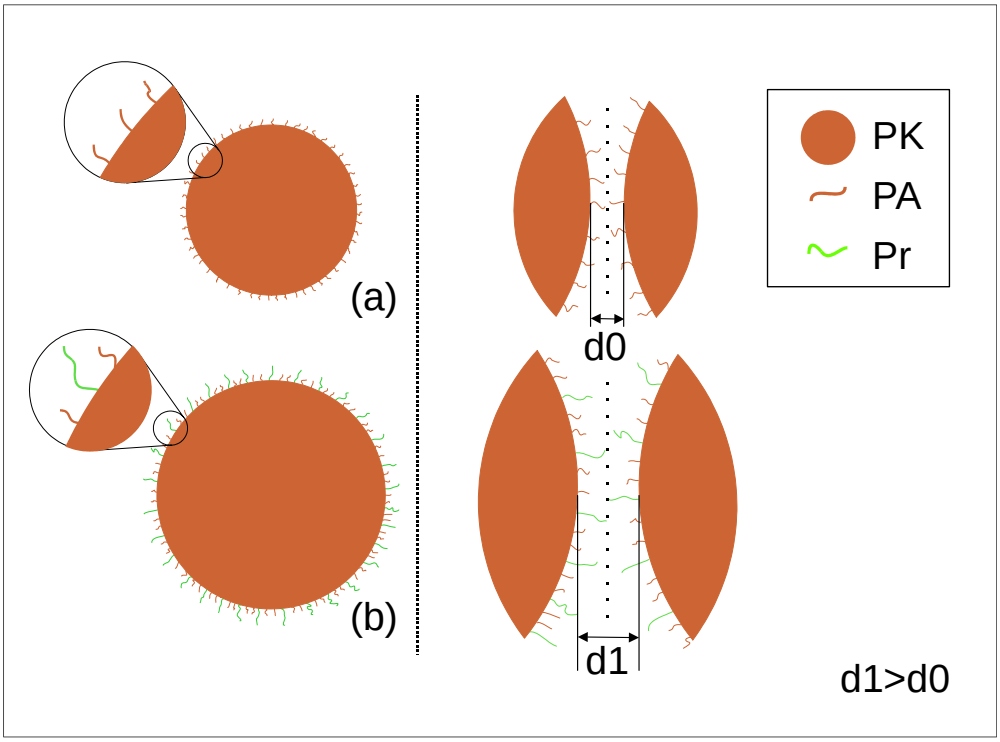

Figure 3.11. Schematic representation of particle structures for the binary system (a) and for the ternary one (b).

For the binary system (*Figure 3.11-(a)*) the protonated polyamine (mPK30), adsorbed at the surface of the polyketone particles, provides stabilization to the system by both electrostatic interaction and steric hindrance. In particular, the latter mechanism is very important at relatively high solids content (thus at relatively small average distance between the particles). When two particles approach, steric repulsion between the mPK30 chains is established, thus preventing coagulation/aggregation of the PK30(II) particles. For the ternary system (*Figure 3.11-(b)*) also the protein chains are assumed to be absorbed at the surface of the PK30(II) particles. Calculations using the computer program Molecular Modeling Pro Plus using the zigzag configuration approach, reveal that on average the protein chains (35 nm) are longer than the mPK30 chains (15 nm). As a result, the steric repulsion will become active at distances (d_1) larger than those characteristic of the binary system (d_0), thus providing the system with extra stabilization. Such hypothesis is impossible to verify with particle size analysis since only the water-insoluble moieties are visible, not the soluble chains at the particle surface. The proposed interaction model can explain the formation of stable emulsion formation, in the presence of proteins, even at relatively low solids contents (the steric repulsion is active at relatively larger distances) and the increase in viscosity upon protein addition with respect to the binary system (Pr chains form entanglements with each other as demonstrated by the non-Newtonian behavior of viscosity in *Figure 3.10*).
The proposed interaction model structure might also explain the difference in viscosity for the two addition protocols. Simultaneous addition of PK30(II) and protein results in higher viscosity than in the case of first adding the protein and then the PK30(II). If the water soluble protein is added before the PK30(II) it might form aggregates with itself or with the surfactant. Such aggregates should be then reversed/fragmented to single protein chains that can further migrate from the bulk of the solution to the surface of the PK30(II) particle and once there can establish entanglement with each other. On the other hand, when the two components are added together, they are already in the most

favorable conditions for the protein to interact with (being absorbed by) the PK30(II) particle.

3.3.1 Wood adhesive tests

The wood panels were prepared by applying the emulsions at 150 g/m^2 single adhesive line onto one side of $(25 \times 50 \times 4)$ mm^3 maple veneer pieces. Then the wood panels were hot-pressed at 200 ºC for 5 min under constant pressure of 3 MPa. For the bond quality as wood adhesive, the glued wood panels were immersed in boiling water and allowed to swell in all directions. The glue has to withstand all the resulting forces. The samples preferably should contain residues from the opposite veneer. This was indeed the case, indicating that the adhesive bond is stronger than the wood (*Figure 3.12*).

Figure 3.12: Sample after shear strength test (surface contains fibers from the opposite veneer wood).

According to the European Standard, the shear strength should be higher than 1 MPa. All adhesives passed the EN-314 norm with higher shear strength than required. *Figures 3.13* and *Figure 3.14* show that the addition protocol and the solids content had no significant effect on the shear strength.

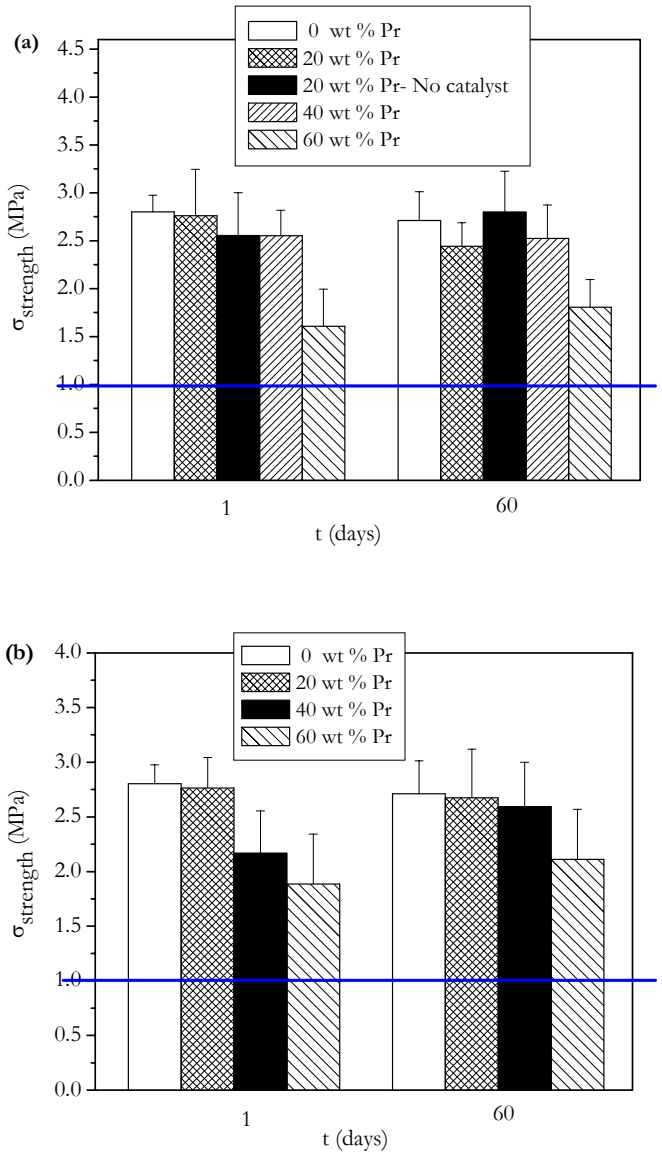

Figure 3.13: Effect of protein intake, presence of catalyst, and storage time on the shear strength of wood adhesive of 50 wt % total solids contents samples with two addition protocols. Horizontal lines represent the minimum values required for passing the test. (a): protein is added together with the PK30(II). (b): protein is added before the PK30(II).

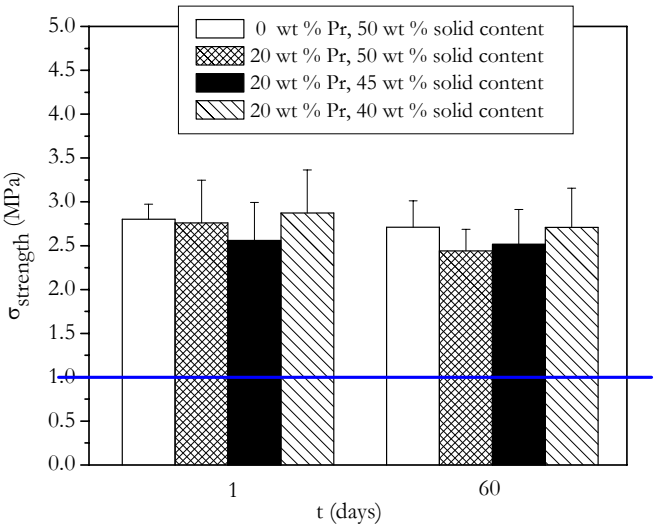

Figure 3.14: Effect of solids content and storage time on the shear strength of wood adhesives. The horizontal line represents the minimum value required for passing the test.

However, the protein intake (*Figure 3.13*) had a clear influence on the emulsions performance as wood adhesives. At 60 wt % protein intake the shear strength is considerably lower than for the adhesives with lower protein contents. This is not surprising if one takes into account that adhesives based solely on soy proteins display, contrary to those based on polyketones, a very weak water resistance [16,18]. It must be stressed however that despite this limitation, the adhesives with 60 wt % protein intake still displayed a shear stress superior to that required by the European Standard. Furthermore, it is remarkable (*Figure 3.13*) that similar shear strength was obtained from the samples without the addition of the curing catalyst (salicylic acid). The role of the latter for adhesives based solely on polyketones [4] consists of promoting the reaction of the carbonyl groups on the polyketone with non-protonated amino groups on the surfactant [4]. The fact that a similar performance can be obtained without any catalyst present in the system might leads to the hypothesis that the acidic parts in the soy protein (mainly aspartic and glutamic acid, present respectively as 13.31 %, 28.25 % of all amino acidic residues, see experimental part) might act as the catalyst in the curing reaction in the hot press. Finally, the samples were tested for adhesive wood performance after two months storage time (*Figure 3.13* and *Figure 3.14*). Similar shear strengths compared to the freshly prepared ones were obtained. This clearly highlights the long shelf-life of the prepared protein-containing polymeric emulsions.

3.3.2 Confocal Fluorescence Microscopy

With the use of confocal fluorescence microscopy, morphological information about the penetration of the glue into the wood [24] could be obtained. In previous studies it was shown that polyamines (i.e. the surfactant in our formulation) exhibit fluorescence properties in aqueous solutions [9]. Soy protein showed also fluorescence properties both in solid and liquid solution (*Figure 3.15*).

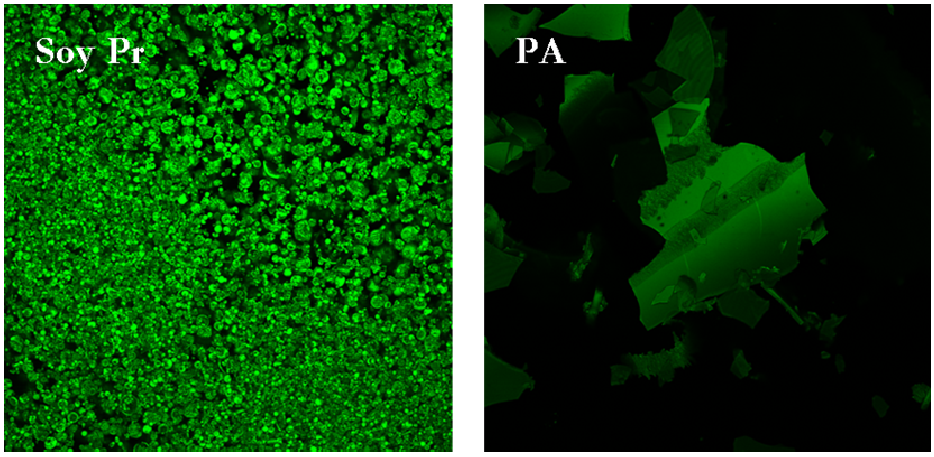

Figure 3.15: Fluorescence microscopy of soy protein and polyamine.

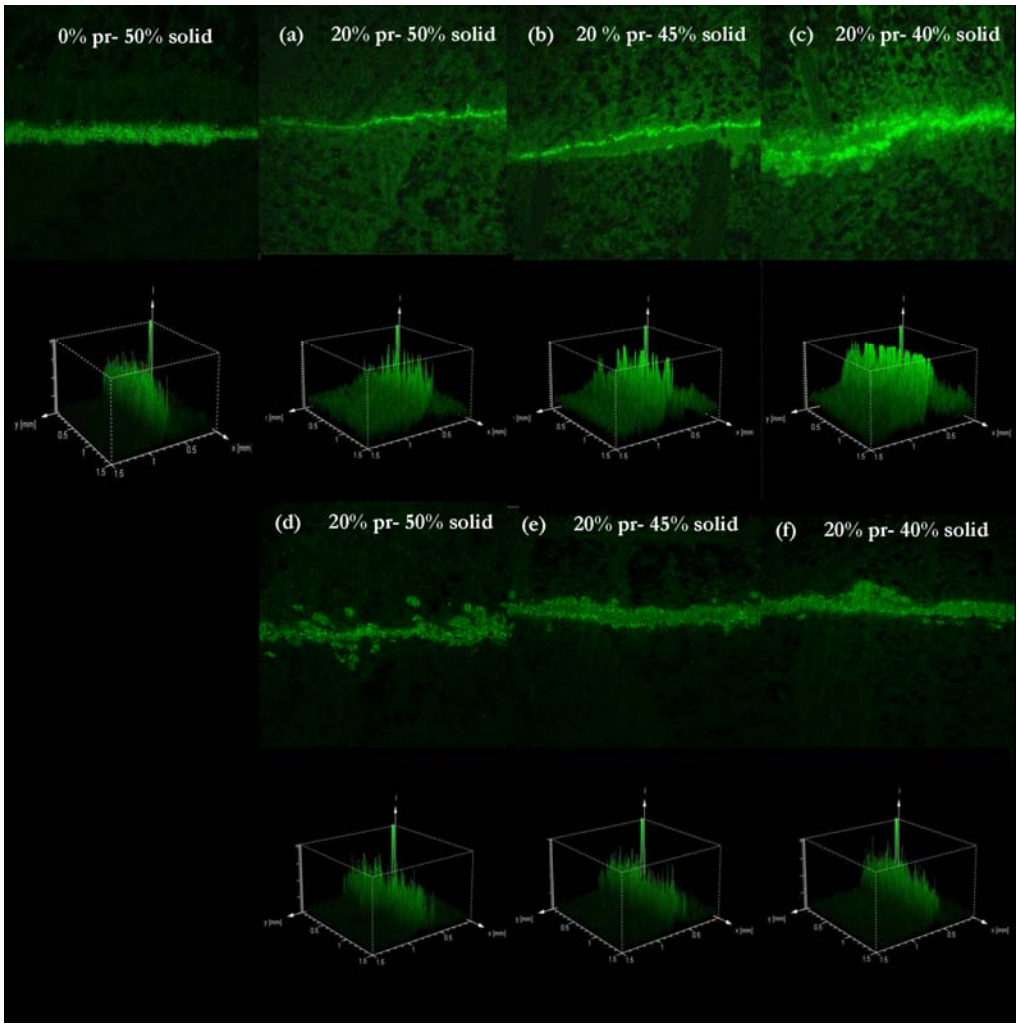

Figure 3.16: Confocal fluorescence microscopy of reference and sample 20 wt % protein at different solids contents, (a, b, c): protein is added together with the PK30(II). (d, e, f): protein is added before the PK30(II).

In the prepared wood panels, small glue lines were observed for the samples with 50 wt % solids content, while samples with 45 and 40 wt % solids contents showed broader glue lines and higher intensities (*Figure 3.16*). This is more obvious in the 3-D views indicating more penetration of the glue into the fibers of the wood.
Just by looking at the concentration profiles of the fluorescent moieties as function of position (i.e. as function of the depth reached into the woods), it can be observed how the penetration depth improves significantly at lower solids contents and, more importantly, when proteins are used in the overall formulation. The first effect can be reasonably explained by the presence of more polar water molecules (at lower solids content) in the formulation, which obviously favor the compatibility of the adhesive with the polar wood surface. Also the positive influence of the protein can be explained by similar reasoning: the water soluble soy proteins have higher affinity for the wood surface than the apolar polyketone chains. Thus, when proteins are present in the basic formulation, a deeper penetration into the wood can be expected (and indeed is observed) simply because the adhesive displays an improved affinity/compatibility with the wood surface. Comparing the two addition protocols, it can be seen that the penetration in the case of the sequential addition (i.e. Pr is added before the PK30(II)), is slightly lower. This might be explained as the following: when the Pr is added before the PK30(II), it forms a complex with the polyamine, this will therefore retard/delay the Pr to migrate to the surface and hence less penetration in the bulk of the wood is expected.

3.4 Conclusions

Aqueous protein-containing polymeric emulsions from chemically modified thermosetting aliphatic polyketones were prepared in a one-pot process, by adding a given amount of soy protein to a polyketone based emulsion. Several factors affecting the production, stability and performance of the emulsions as wood adhesives were studied. Emulsions containing proteins could be prepared at 45 and 40 wt % total solids contents, a composition not possible to achieve by just using a polyketone. The resulting emulsions were stable for at least two months at ambient temperature. The average particle size of the emulsions containing proteins was in almost all cases higher than the reference adhesive with no protein added except for the sample with 60 wt % protein intake from the sequential addition protocol. The viscosity of the protein-containing emulsions was higher than the one of the reference sample (no protein added) and it decreased in time. In agreement with these trends and with the results as a function of the addition protocol, a possible explanation of the role of the protein in the emulsion is proposed but needs further study (currently performed) to be confirmed.
Form a practical point of view, all the prepared protein-containing emulsions passed the European standard (EN-314) wood test with higher shear strength than required. The presence of up to 40 wt % proteins in the basic adhesive formulation results in a slight increase of the average particle size as well as of the viscosity. This protein intake represents a kind of optimum for this system, thus allowing to substitute as much as possible of the unmodified PK with proteins and still retaining the same emulsion structure and stability as well as wood-adhesive performance. Furthermore, broader glue lines (improved glue penetration in the wood) were observed in the presence of proteins (i.e. with respect to the reference polyketone-based adhesive) in particular for systems with low solids contents, which can be prepared only when using proteins in the basic formulation.
The main advantage of using soy protein in the polyketone-base formulation is that the properties and performance as wood adhesive are not changed and that they could be prepared at different solids contents. Besides, the use of soy proteins is an advantage

from economic point of view as it is low-cost filler. Research on the application of different types of proteins as well as other biomaterials in the polyketone-based wood adhesive is ongoing.

3.5 Abbreviations

PK30(II): second amount of polyketone 30 mol % ethene content, unmodified polyketone
mPK30: modified polyketone 30 mol % ethene with 1,2-diaminopropane, Polyamine
PK: Polyketone
Pr: Soy protein
rpm: revolution per minute
min: minute
wt %: weight percentage
$\bar{d}$: The average particle size of the emulsions (nm)
d(H): the hydrodynamic diameter (nm)
k: Boltzmann's constant (J/K)
T: the absolute temperature (K)
D: the diffusion coefficient (m^2/s)
η: viscosity (Pa.s)
$\dot{\gamma}$: shear rate (s^{-1})
$\sigma_{strength}$: shear strength (MPa)
d_0: overlapping distance between the PA chains (binary system)
d_1: overlapping distance between the PA and the Pr chains (ternary system)

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Chapter 4: Soy proteins in polyketone-based wood adhesives: mechanistic insight

Abstract

Chemically modified low molecular weight polyketones in combination with their unmodified precursors can be used as wood adhesives. This work describes a systematic study on the preparation of emulsions of chemically modified polyketones containing soy protein in a one-pot process. The effect of the soy protein and unmodified polyketone intake with respect to the intake of chemically modified polyketone is investigated in a "matrix-like" experimental design study at a total specified solids content of 45 % wt. The stability and shelf-life of the emulsions as well as the performance as wood adhesive were studied at room temperature. The results were modeled using multiple linear regression ($R^2 \geq 0.961$) to predict the shear strength 1 day after the emulsion preparation ($\sigma_{1\ strength}$). All the prepared emulsions were qualified as wood adhesives according to the European Standard EN-314.

Further insight into the role of the soy protein on the stability and performance of the emulsions is obtained by surface tension measurement. The protein chains appear to act as a co-surfactant and thickening agent.

4.1 Introduction

Conventional wood adhesives derived from formaldehyde based resins such as urea-formaldehyde (UF) and phenol-formaldehyde (PF) [1-6] are known to release formaldehyde to the environment during production and upon curing. According to the World Health Organization, formaldehyde is suspected to be carcinogenic and harmful to the environment [1,4-7]. Increasing environmental concerns require the development of safe and non-toxic wood adhesives [4-8].

The use of aqueous polymeric emulsions containing polyketones in combination with amine-modified polyketones (i.e. polyamines) has been reported as excellent wood adhesives [1]. The emulsion is prepared in a two-step procedure (*Figure 4.1-(I)*).

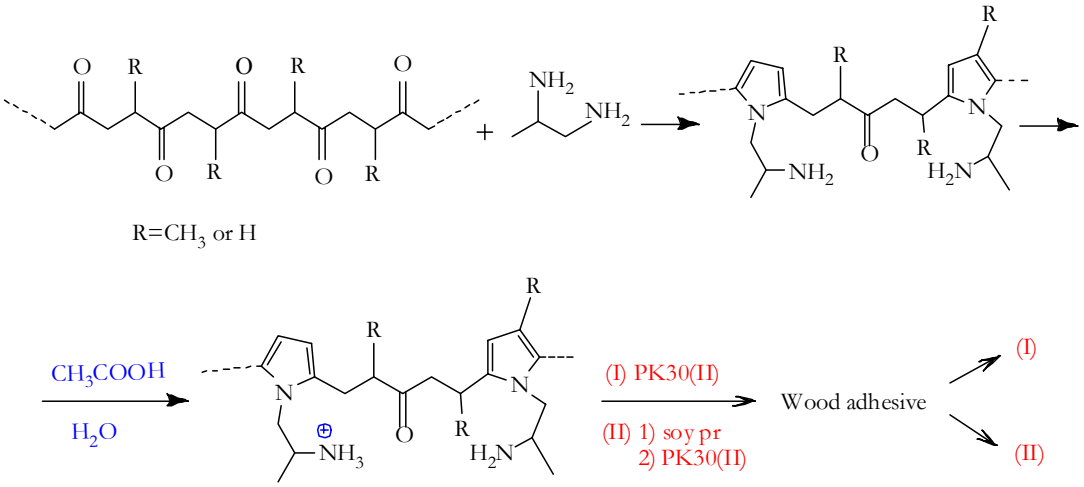

Figure 4.1: Preparation of the protein-containing aqueous emulsions

In this case the polyketones are reacted with 1,2-diaminopropane (1,2-DAP) to prepare polyamines. The polyamines have a double functionality, a pyrrole ring and an amino functional group, which are converted to water-soluble cationic compounds by protonation with weak acid (acetic acid). These polyamines, now acting as a polymeric surfactants, were used to prepare the water-based polyketone emulsions by addition of a second amount of polyketones *(Figure 4.1-(I- Wood adhesive (I)))*.

It is of interest to replace part of the active components in the polyketone based wood adhesives by renewable and low cost alternatives. Soy protein is considered an attractive alternative to synthetic polymers in adhesives, plastics and binders. It is a readily available renewable source, inexpensive, and able to bind wood at relatively high moisture contents. Furthermore, soy proteins can be processed in cold and hot press conditions [4,5,8,9]. Unfortunately, adhesives made from soy protein showed high water sensitivity, low shear strength and suffer from biological degradation [8]. However, in combinations with polyketone-based adhesives good quality wood adhesives may be prepared (*Figure 4.1-(II-Wood adhesive (II))*) [10].

The functional properties of soy proteins are a strong function of their structure in solution [11,12]. Soy proteins in water solutions are considered to be highly ordered, with hydrophilic groups at the outside and the hydrophobic groups inside the structure [12,13]. This distribution of the hydrophilic and hydrophobic groups in a protein is expected to have an effect on protein-substrate interactions. As such, it is also likely to have an effect on glue strength and emulsion rheology when used as an additive in polyketone based wood adhesives [10,12,14]. A great deal of research has been carried out in the food industry to develop a better understanding of the functional properties of proteins in food emulsions. It is generally acknowledged that proteins play a role in the

stabilization of the emulsion as well as in emulsion formation, very much like the action of a "classic" surfactant [15,16]. Unfortunately, such studies have not yet been published for the role of proteins in polyketone based wood-adhesive emulsions. Despite the similarity, the variety in the possible chemical structures of the components in the formulation, in particular of proteins, makes a direct extrapolation of the acquired knowledge practically impossible. Furthermore, the use of relatively low molecular weight proteins (fully soluble in water), as in the present work, complicates the research for a general concept since these proteins do not behave as surfactants.
In this work, two factors that are known to have an effect on the preparation and stability of the polyketone-based emulsions containing soy proteins were systematically investigated. The ratio unmodified polyketone to amine-modified polyketone (polyamine) as well as the ratio protein to polyamine were systematically varied. All emulsions were tested as wood adhesive according the European EN-314 Standard for wood adhesives. Multiple linear regression was used to predict the shear strength 1 day after the emulsion preparation as a function of various emulsion component intakes. Furthermore, the role of soy protein in the emulsion was investigated.

4.2 Experimental part

4.2.1 Materials

Polyketone with 30 mol % ethene based on the total olefin content (PK30, M_w 2670) was synthesized according to a reported procedure [17]. 1,2-Diaminopropane (1,2-DAP, 99+ %, Acros), Acetic acid (99.5 % pure, Acros), Salicylic acid (reagent ACS, Acros), Soy Protein acid Hydrolysate (Soy Pr, Sigma-Aldrich, Mw between 5500-7000 Da measured using MALDI-TOF). n-Butylamine (99.5 %, Acros), n-Hexylamine (≥ 98 %, Fluka), n-Octylamine (≥ 98 %, Fluka), and n-Dodecylamine (≥ 98 %, Fluka), were purchased and used without further purification. Commercial wood maple veneers were purchased from Sikkens Center Groningen (The Netherlands). Double distilled water was used in all experiments. Amino acid composition analysis on the soy protein was performed by "Eurosequence B.V Analysis and Synthesis of Protein and DNA", Groningen, the Netherlands.

4.2.2 Emulsion preparation

Polymeric amines (PA), acting as surfactants in the final formulations, were first prepared (*Figure 4.1*) by chemical modification of the polyketone with 1,2-DAP to deliver mPK30. The reaction was carried out according to a well-known procedure [18] in a 250 ml rounded bottom glass reactor with a reflux condenser, U-type anchor impeller and an oil bath. First the polyketone (40.0 g, 0.304 mol; calculations based on dicarbonyl units in the PK30 polymer) was heated to a temperature of 100 ºC, then 1,2- DAP was added drop-wise (18.02 g, 0.243 mol, based on initial molar ratio between 1,2-DAP and the carbonyl groups in the PK of 0.8) during the first 20 minutes of the reaction. The stirring speed was kept constant at 500 rpm. The reactant mixture changed from yellowish to brown and became a solid material upon cooling to room temperature. The prepared polymeric amines were washed several times with double distilled water, filtered and freeze-dried. The final product was a light brown powder. The conversion of the carbonyl groups to pyrrole rings were determined by using elemental analysis, and found to be around 70 %. These polyamines are converted to water-soluble cationic compounds by protonation with acidic acid solution in double distilled water to match a desired protonation level of 50 %. In the emulsification step (*Figure 4.1-(II)*), a given amount of soy protein (Pr) was added, followed after one hour by addition of a second

amount of unmodified polyketone PK30(II). After another hour, double distilled water was added to reach the desired 45 wt % solid content. All synthetic steps were performed in a one-pot process. The resulting emulsions were stored at room temperature in sealed high-density polyethylene (HDPE) containers and further analyzed.

Different factors affect the production, stability and performance of the emulsions as wood adhesives. These include rotor speed, emulsification time and temperature, protonation level of the polyamine (mPK30), chemical structure of the polyketone and the addition protocol [1,10]. In the present work all these factors have been fixed (*vide supra*) to values previously reported [10] while the overall chemical composition expressed as PK30(II)/mPK30 and Pr/mPK30 wt ratios, r_1 and r_2 respectively in the text) has been systematically varied according to a "matrix-like" experimental design (*Figure 4.2*).

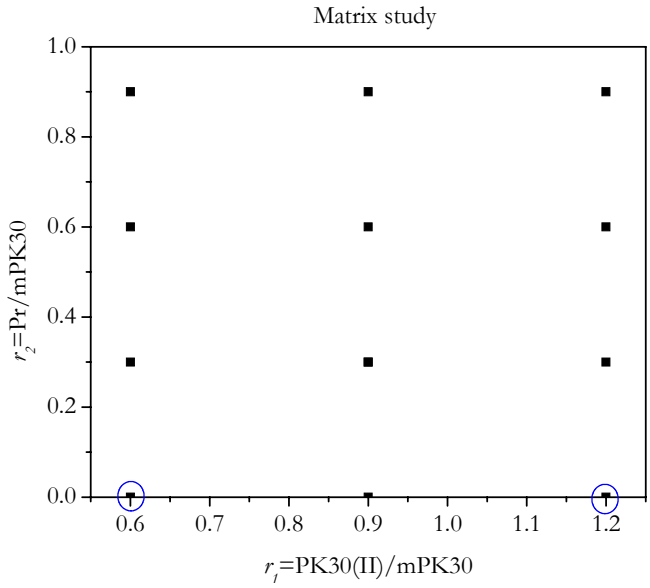

Figure 4.2: Experimental design for preparations of soy protein based polyketone emulsions, circles on points represents samples that could not be prepared.

In Table 4.1, a chemical composition of all prepared emulsions per 1 g polyamine present as surfactant is presented.

Amount of mPK30, (g)	Amount of Pr, (g)	Amount of PK30(II), (g)	r_1=PK30(II)/mPK30	r_2=Pr/mPK30
1	0	0.6	0.6	0
1	0	0.9	0.9	0
1	0	1.2	1.2	0
1	0.3	0.6	0.6	0.3
1	0.3	0.9	0.9	0.3
1	0.3	1.2	1.2	0.3
1	0.6	0.6	0.6	0.6
1	0.6	0.9	0.9	0.6
1	0.6	1.2	1.2	0.6

1	0.9	0.6	0.6	0.9
1	0.9	0.9	0.9	0.9
1	0.9	1.2	1.2	0.9

Table 4.1: Chemical compositions of all prepared emulsions.

Reference sample in the absence of soy protein at a ratio r_1 of *0.6* resulted in an emulsion with a viscosity exceeding the limits of the viscosity measurement instrument, and hence this sample was excluded from this study. The reference sample without proteins at a ratio r_1 of *1.2* could not be prepared as immediate phase separation during the preparation was observed. Therefore, only the reference sample at a ratio r_1 of *0.9* was taken as a comparison in this study.
To simplify the matrix study, horizontal and vertical comparisons were made. The first comparison was by fixing the ratio of r_1 and varying the ratio of r_2 (vertical comparison). The second comparison was by fixing the ratio of r_2 and varying the ratio of r_1 (horizontal comparison).

4.2.3 Rheological analysis

The emulsion viscosity (η) was measured at 20 ºC by using an AR 1000 Rheometer (TA Instruments, USA). The aluminum cone-and plate fixture was 2º cone angle and 40 mm cone diameter. The apparent viscosity of the samples was measured at a constant shear rate ($\dot{\gamma}$) of 15 s^{-1} for 45% wt solids content samples. The viscosity-shear rate relationship was established by measurement at different shear rates in the range from 5-60 s^{-1}.

4.2.4 Wood adhesive testing

The wood pieces of maple veneer for the adhesive tests were dried at 105 ºC for 10 h to reduce the moisture content to a constant level. A given amount of salicylic acid (0.5 % wt, based on the sum of the intake of the second amount of polyketone and the soy protein) was used in the emulsion as a curing catalyst.
The emulsions were applied at a level of 150 g/m^2 using a single adhesive line onto one side of a maple veneer piece. According to a reported procedure [1,10], the area where the glue was exposed on every piece was $(25*25)$ mm^2. The specimens were hot-pressed for 5 minutes at 200 ºC at a constant pressure of 3 MPa. Ten to thirteen replicates were tested for each experiment. The bond quality was tested according to the European EN-314 Standard test. Before analysis, the specimens were first immersed in boiling water for 72 hours and then cooled to room temperature in water for at least one hour. The shear strength ($\sigma_{strength}$) was measured by using an Instron 4301 machine using 5 KN power sensor with a crossing speed of 2 mm/min.

4.2.5 Drop tensiometry

Surface tension measurements were performed using a drop volume tensiometer (Lauda-TVT1), equipped with a Lauda RM6 temperature controller. The measurements were performed at a controlled temperature of 25 ± 0.1 ºC. The inner radius of the capillary was 1.055 mm and the volume of the syringe was 500 µL. The sensitivity (instrumental error) for interfacial tension was 0.1 mN/m [19]. The surface tension value for double distilled water (71.98 mN/m) was measured and taken as standard before the measurements were performed [20].

4.2.6 Mathematical analysis and Modeling

The mathematical analysis of the experimental data was performed using "MathCAD 14" (Mathsoft) software package.

4.3 Results and Discussion

4.3.1 Structure-property relationships

In our previous study [10] on the use of soy proteins in PK-based wood adhesives different amounts of soy protein (20, 40, 60 wt % with respect to the unmodified polyketone, PK30(II)) were co-formulated with the polyamine (surfactant, mPK30) and PK30(II) by keeping the weight ratio between the soy protein plus the unmodified polyketone to the polyamine equal to 1.5 wt/wt.

$$r = \frac{\text{amount of proteins} + \text{amount of unmodified polyketone}}{\text{amount of polyamine}} = 1.5 \qquad (4.1)$$

Such experimental plan finds its conceptual origin on the initial assumption that the added proteins might simply act as dispersed phase (filler) in the emulsion, thus in a role comparable to the one of the unmodified polyketone. However, our previous study [10] clearly indicated a significant role of the protein as co-surfactant in the system. Therefore, in the present work, a systematic study with a broader range of variables has been performed by independently changing two ratios: the one between the unmodified polyketone to the polyamine (r_1), and the one between the soy protein to the polyamine (r_2).

First, the stability of the final emulsions was studied as a function of storage time at 45 % solids content using rheology analysis at 20 ºC. Typical viscosity profiles are given in *Figure 4.3* and are characterized by a decrease in viscosity as a function of time. The overall trend is in agreement with our previous results [1,10]. This can be explained through the proposed hypothesis that this decrease is caused by polymer rearrangement/relaxation within each particle. The samples at the beginning reveal non-Newtonian behavior and change over time into Newtonian. This shear thinning is typical for polymeric systems where the chains form entanglements with each other [1], initially as stretched surfactant chains at the surface and then in time partially retracting closer to the surface of the particles (transition to Newtonian behavior).

The viscosity is a clear function of the two ratios, r_1 and r_2. In a "vertical" comparison (*Figure 4.3,* data at *r1=1.2* is not shown for brevity) at fixed r_1 values, the viscosity trend was the following: $\eta_{r2=0.6} < \eta_{r2=0.3} < \eta_{r2=0.9}$. The viscosity first decreased as the protein intake increased (r_2 values) and then increased again thus indicating the presence of a minimum viscosity as a function of r_2. The viscosity increase by going from $r_2 = 0.3$ to 0.6 is in agreement with a thickening effect of the soy protein in the emulsion. This is in turn probably the result of increased intermolecular interactions due to unfolded protein molecules. The major forces that facilitate such interactions are electrostatic and covalent disulphide bonding [8].

At higher r_2 values *(> 0.6)* the viscosity decreases however again probably as result of protein complexes formation at relatively high protein intakes.

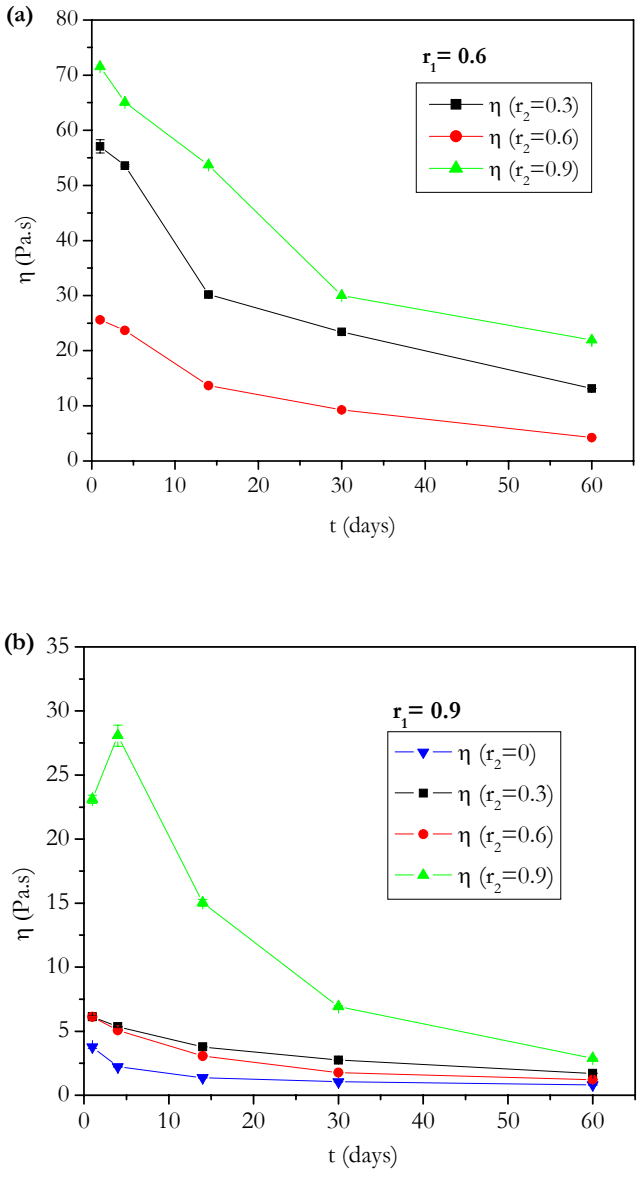

Figure 4.3: Viscosity of the polyketone-soy protein emulsions as a function of storage time. (a): at a fixed r_1=*0.6* as function of r_2. (b): at a fixed r_1=*0.9* as a function of r_2. T = 20 ºC.

When considering "horizontal" comparisons (i.e. at fixed r_2 values) in our matrix-like experimental design (*Figure 4.4*, data at r_2=0.3 not shown for brevity), the viscosity trend is the following: $\eta_{r1=0.6} > \eta_{r1=0.9} > \eta_{r1=1.2}$. In this case, no minimum viscosity is observed instead a monotonous decrease with an increase in r_1 values is found. This is also in agreement with our previous study [10], which already established a decrease in the emulsion viscosity as a function of the PK intake.

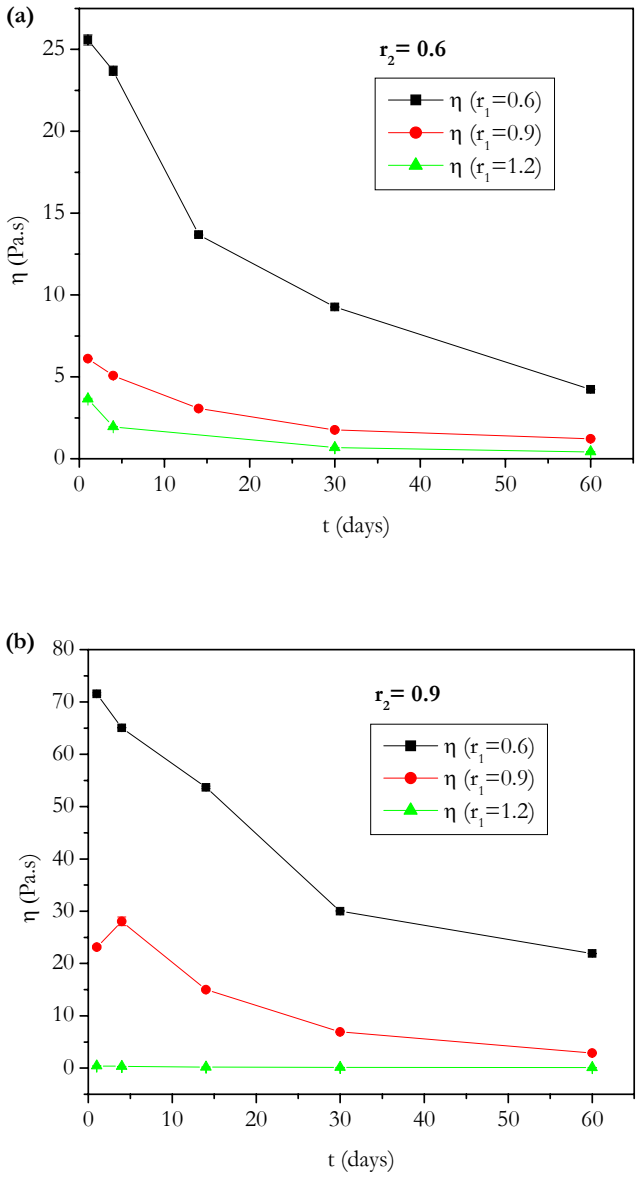

Figure 4.4: Viscosity of polyketone-soy protein emulsions as a function of storage time. (a): at a fixed r_2=*0.6* as function of r_1. (b): at a fixed r_2=*0.9* as a function of r_1. T = 20 °C.

All prepared emulsions were tested as wood adhesives according to the European EN-314 Standard test. The results (*Figure 4.5*) clearly showed that the prepared emulsions passed the EN-314 test with higher shear strength than required (1 MPa as a minimum required value). Good and comparable results were obtained also after 60-days of storage time at room temperature.

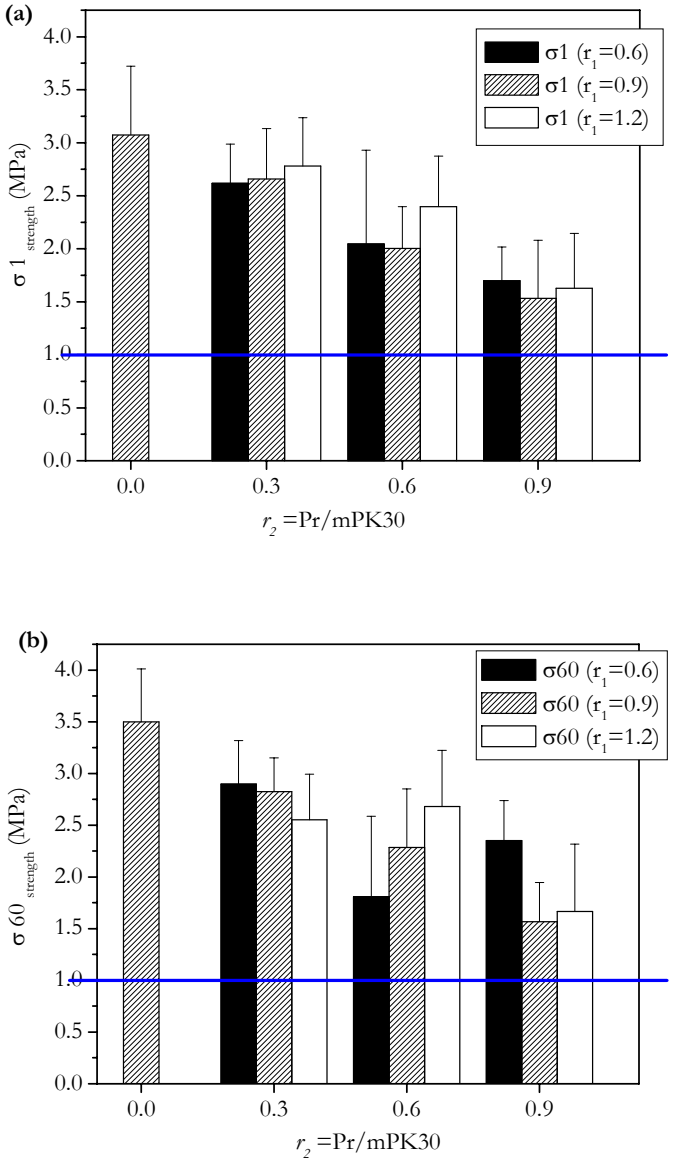

Figure 4.5: Shear strength of the samples from the matrix study. (a): shear strength after 1-day storage time. (b): shear strength after 60-days storage time. The horizontal line represents the minimum value required for passing the test.

By comparing the shear strength of the samples at fixed r_1 values (e.g. black bars in *Figure 4.5*), it is seen that higher protein intakes result in significantly lower shear strengths. This is not surprising since adhesives based only on soy protein emulsions exhibit weak water resistance [8]. In addition, too high protein intakes resulted in increased viscosity, which in turn led to poorer application/spreading of the adhesive on the wood veneer. Furthermore, it is known that a lower adhesive viscosity enhances the penetration in the porous structure of the wood because of the ease of spreading [8].
On the other hand, at fixed r_2 values (*Figure 4.5*, individual bar groups), the amount of PK in the formulation does not have a significant effect on the performance. The observed invariance of the shear strength as a function of the amount of PK suggests

that a saturation limit is already achieved at the lowest PK30(II) intake (thus no further improvement in the adhesion observed when adding more PK30(II)). A further possibility is a change in the adhesion mechanism from chemical reaction towards physical/hydrogen bonding adhesion as demonstrated for glues based on proteins only [21,22]. However, this can be partially ruled out since one would expect a decrease, rather than an invariance of the shear strengths values as result of the boiling procedure in water for 72 h. From a product application point of view, the use of relatively low PK intakes in the formulation provides a simple way to retain good performance at relatively lower costs.

A statistical model [23] was used to obtain correlations between the shear strength after 1 day $\sigma_{1\ strength}$ (dependant variable (y)), and the chemical composition of the emulsions (i.e. r_1 and r_2). The resulting model has the following form:

$$y = 2.932 + 0.247r_1 - 1.687r_2 \qquad (4.2)$$

The suitability/validity of the model was checked by the analysis of variance (ANOVA, Table 4.2). The test procedure [23] included partitioning the total sum of squares into the sum of squares (*SS*) for the model and the sum of squares for the error. From the sum of squares, it is then possible to calculate the mean square (*MS*) and the error, knowing the degrees of freedom (*DF*) for the system.

	SS	DF	MS	F	P-value	R^2 values	
Model	2.493	2	1.247	98.506	2.319×10^{-6}	R^2	0.961
Error	0.101	8	0.013			$R^2_{adjusted}$	0.956
Total	2.595	10				R^2_{press}	0.914

Table 4.2: Analysis of variance (ANOVA) for the statistical model.

The resulting P-value (the probability that the model is due to a random noise of the experimental points) is very low and ensured that the model was statistically significant. The validity of the model is further confirmed by the R^2 values (Table 4.2). The R^2 value for the model is 0.961 and the adjusted-R^2 is 0.956. The two values are very close to each other indicating that no significant variables are left out of the model [23]. The model validity is further confirmed by the parity plot (*Figure 4.6*), showing very good agreement between experimental and predicted values.

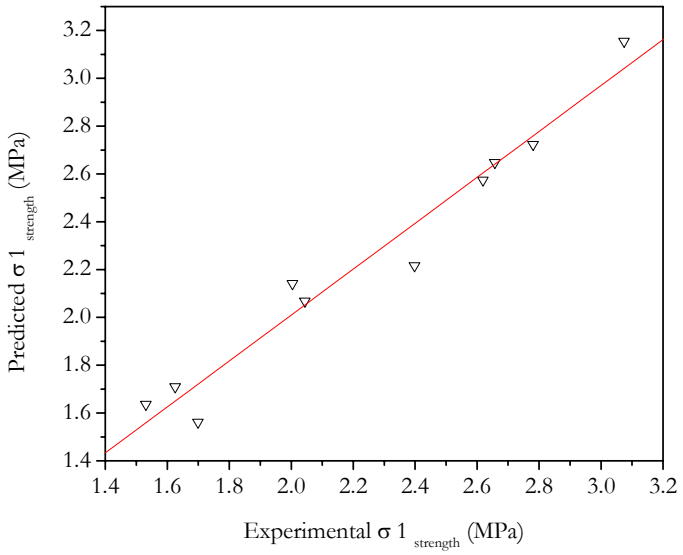

Figure 4.6: Plot of predicted vs. experimental values

A prediction of error sum of squares analysis (PRESS analysis) [23] was also performed as it constitutes an internal validation method for the model. From the value of R^2_{press} 0.914 it can be seen that the model correctly predicts the shear strength after 1 day ($\sigma_{1strength}$) as a function of the independent process variables.
Thus, the developed model may be used as a tool to predict the shear strengths as a function of the chemical composition of the emulsion. Moreover, the sign and magnitude of the coefficients in equation 4.2 clearly confirm that r_1, i.e. the amount of unmodified PK in the formulation, has only a slight influence on the final performance (at least within the experimental range studied here) while r_2, i.e. the amount of proteins in the formulation, has a significant (negative) influence on the shear strength. As a consequence one must conclude that the amount of protein to be used should be carefully chosen in order to combine positive effects on emulsion stability and viscosity (*vide supra*) and at the same time retain a positive performance during the wood adhesion test.

4.3.2 Role of protein in emulsion

From the discussion above, it is clear that proteins affect the structure of the emulsions as well as their performance as wood adhesives. To gain a better insight into the role of the proteins in the formulation, we first investigated the surface activity of the virgin soy protein. For comparison, the surface tension of the modified polyketone (polyamine) as well as that of simple low molecular weight amines at 50 % protonation level were determined (*Figure 4.7*). The pure soy protein, which is soluble in water, does not show any surface activity in the range of concentrations used. As expected [18], the polyamine reduces the surface tension, a fact related to its macromolecular structure with hydrophilic and hydrophobic moieties along the backbone. The surface tension of the low molecular weight amines is clearly a function of the length of the carbon chain. For the longer chains (octylamine and dodecylamine), the drop in surface tension becomes more relevant at higher concentrations and at different protonation levels (50, 70 and 90 %; only the data of 50 % protonation is shown here for brevity). Butylamine does not

show any activity, in agreement with the presence of a very short hydrophobic chain in the molecule.

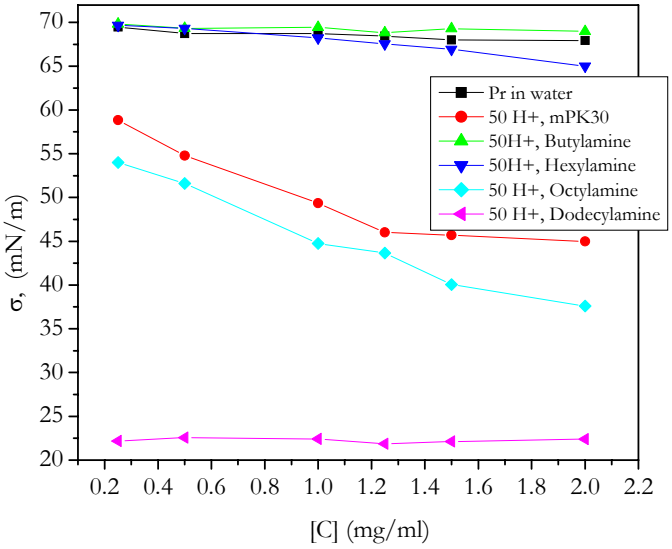

Figure 4.7: Surface tension of low molecular weight amines, mPK30 and soy Pr in water solutions.

Based on the data above, it is tempting to rule out surfactant properties of the soy proteins. This would however, *a priori* neglect possible interaction of the proteins with other components in the emulsion formulation. Indeed, when the protein is added to the octylamine and to the polyamine solutions, a remarkable different surface activity behavior was noticed (*Figure 4.8*).

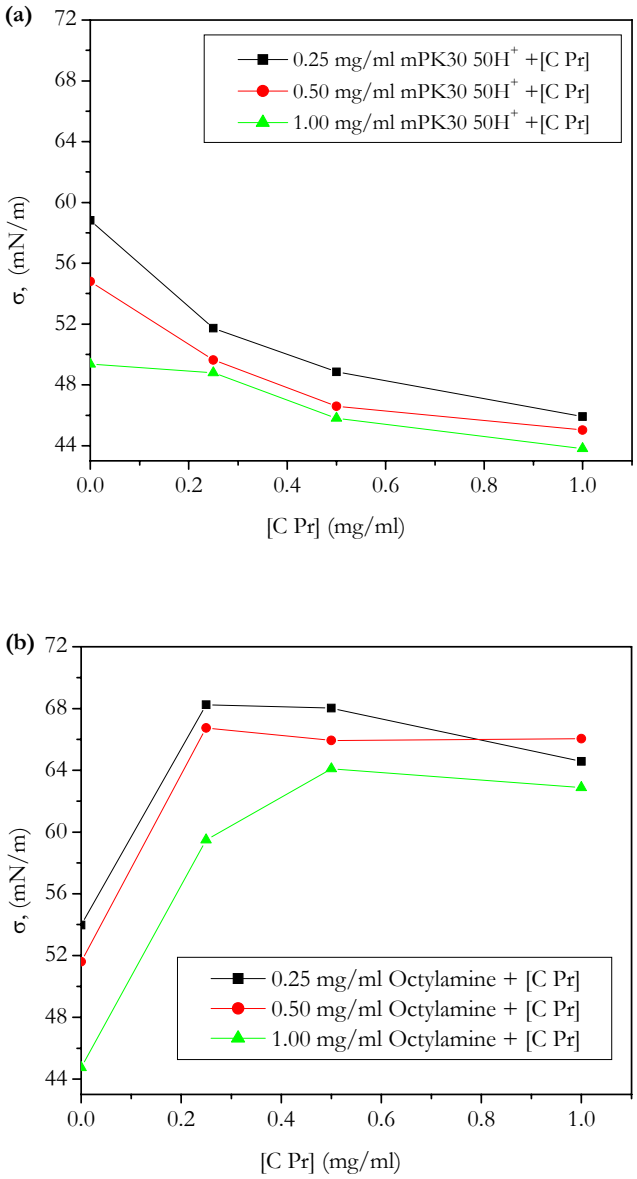

Figure 4.8: Effect of soy protein addition on the surface activity. (a): effect of Pr addition on different concentrations of 50 % protonated mPK30 solutions. (b): effect of Pr addition on different concentrations of octylamine protonated solutions.

Addition of the protein to the polyamine system results in an increase in the surface activity (decrease of surface tension in *Figure 4.8-(a)*). A similar synergetic effect was observed in the literature for gelatin and sodium dodecyl sulfate (SDS) [24]. In the case of alkylamines, (*Figure 4.8-(b)*), adding the protein to the solution results in a dramatic increase in the surface tension. Thus, it seems that the "macromolecular" character of the polyamine, as compared to the low molecular weight amines, plays a decisive role in inducing this synergetic effect. The alkylamines are small molecules and have shorter chain lengths than the polyamine. They are also known to be mobile and surface active at the air-water interface [13]. The protein is bulky in nature and has a high molecular weight (5500- 7000 Da); each protein consisting of long chains of amino acids differing

in the amino acids compositions [8,25]. Normally the protein tends to fold in a coil-like structure in aqueous media so it exposes its hydrophilic groups in water and shield the hydrophobic groups in the center of the coil [12,13]. As a result of these considerations, the following explanations have been proposed for the drop or increase in the surface tension of the amine/protein combinations (*Figure 4.9*):

- when adding the protein to low molecular weight surfactant (protonated octylamine in *Figure 4.8*) the electrostatic interaction (attraction) pulls the amine molecules from the air-water interface back in the solution where the bulky proteins are situated (as deduced from the fact that pure proteins do not show any surface activity);
- when adding the protein to the high molecular weight surfactant (polyamine) precisely the opposite takes place: the polymeric amine is able to pull the peptide chains from the bulk of the solution towards the interface. Therefore, as is the case for most ionic surfactants [26], the new formed complex will still act at the interface as surface-active component.

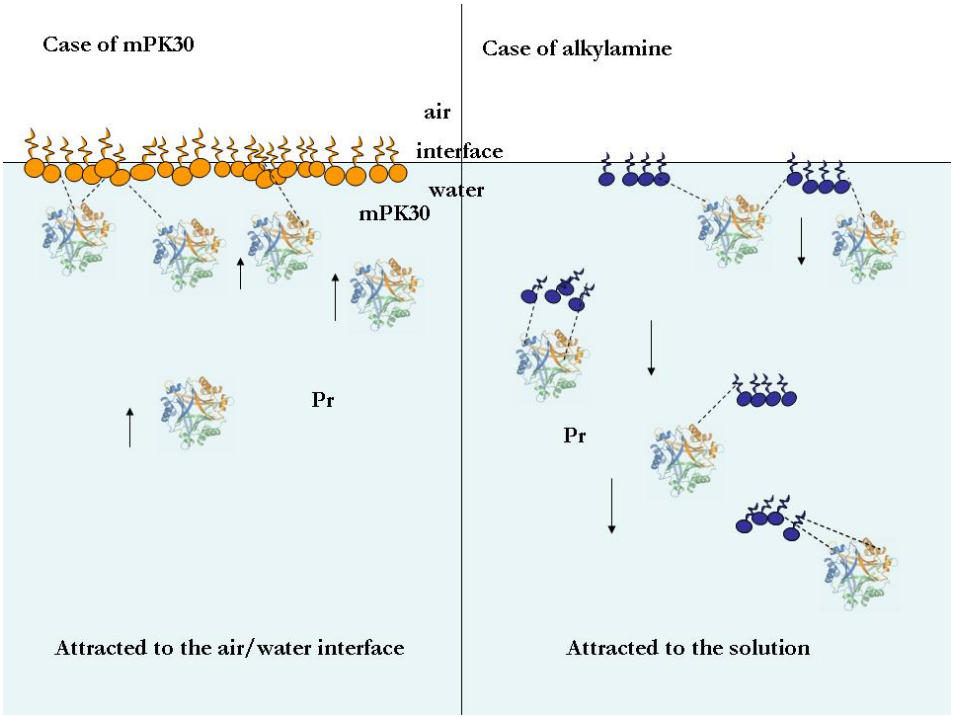

Figure 4.9: Effect of adding soy protein to the mPK30 and the alkylamine chains.

We assumed in first instance that electrostatic interactions are here the driving force for the observed changes. The latter hypothesis is based on the amino acid composition of the protein where roughly 42 % of the acidic groups are present as side chains (see experimental part). Upon dissociation in the water solution, these groups could electrostatically interact with the protonated amines present on the surfactant. However, other kinds of interactions (e.g. hydrophobic) [27] could play a role here.
From our previous results [10] on emulsions with binary systems where only PK and mPK30 were present, it was assumed that the mPK30 would absorb on the surface of the PK to give extra stabilization to the system by both electrostatic interaction and steric hindrance, *Figure 4.10-(a)*. In the ternary system, where PK, mPk30 and Pr are present, assuming that the Pr will absorb at the surface of the PK, *Figure 4.10-(b)*, from previous calculations, it was found that the chains of the Pr are longer than the chains of the

mPK30. Therefore, the steric repulsion will be at distances larger than in the binary system thus providing the emulsion with extra stabilization.
Independently of the exact mechanism of interaction and mechanism of complex formation, one must stress here the fact that the observed behavior was a result of a synergetic interplay between the two different components in the system. The properties and behavior in water solution of protein and polyamine independently does not provide enough information for a full understanding of the system. Moreover, the observed phenomenon at the air-water interface can also be claimed to happen at the PK-water one (thus in real emulsions).
In this respect absorption of the protein at the PK particle/water interface (through complex formation with the polymeric surfactant) might be held responsible for the presence of the peptide chains at the surface where they can provide extra stabilization to the dispersion via electrostatic or steric repulsion [10, 27]. This can be illustrated as in *Figure 4.10-(c)*. As a consequence, when two particles approach each other (*Figure 4.10-(d)*), a steric repulsion between the complex chains is established, preventing the aggregation of the PK particles and thus providing extra stabilization to the system.

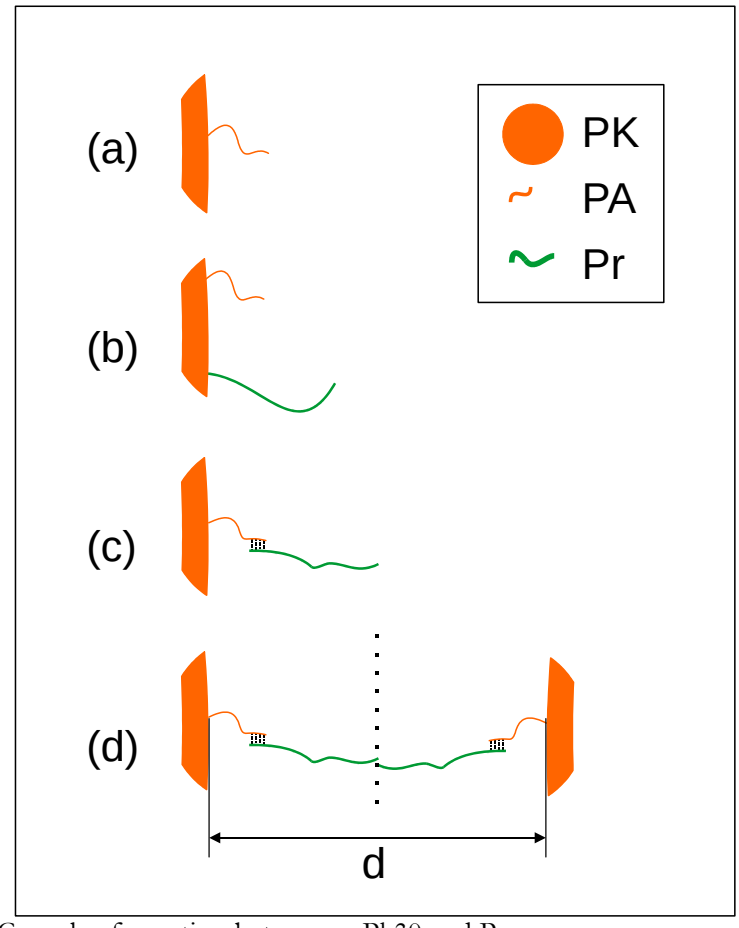

Figure 4.10: Complex formation between mPk30 and Pr.

4.4 Conclusions

Aqueous protein-containing polymeric emulsions based on thermosetting polyketones were prepared in a one-pot process by adding an amount of soy protein to the polyketones based recipe. During the emulsion preparation, two compositional variables

were systematically changed: the amount of unmodified polyketone to the amount of modified one, and the amount of soy protein to the amount of modified polyketone. The viscosity of the protein-containing emulsions was a function of the mentioned ratios, but in all cases was much higher than that of the reference sample (no protein added). This result confirmed our previous study about the role of the protein in the emulsion. Surface tension analysis together with the use of model (low molecular weight) compounds clearly demonstrate complex formation between proteins and the protonated polyamine. This complex is responsible for an increased surface activity of the surfactant and thus for the emulsion stabilization.
All the prepared protein-containing emulsions performed as wood adhesive and passed the European EN-314 Standard. A Significant statistical model with ($R^2 \geq 0.961$ and P-value of 2.319×10^{-6}) using a multiple linear regression analysis was developed to predict the adhesive bond shear strength ($\sigma_{1\ strength}$) for emulsions tested 1 day after preparation..

4.5 Abbreviations

PK30(II): second amount of polyketone 30 mol % ethene content, unmodified polyketone
mPK30: modified polyketone 30 mol % ethene with 1,2-diaminopropane, (polyamine)
Pr: soy protein
PK: polyketone
h: hours
η : viscosity (Pa.s)
$\dot{\gamma}$: shear rate (s^{-1})
σ: is the interfacial tension (mN/m)
r_{cap}: is the radius of the capillary (mm)
[C Pr]: Concentration of Pr (mg/ml)
r: is the ratio between soy protein and unmodified polyketone to the polyamine
r_1: is the ratio between unmodified polyketone to the polyamine
r_2: is the ratio between soy proteins to the polyamine
$\sigma_{1\ strength}$: shear strength after 1 day (MPa)
$\sigma_{60\ strength}$: shear strength after 60 days (MPa)
ANOVA: analysis of variance
SS: Sum of squares
MS: mean square
DF: degree of freedom

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Chapter 5: Extraction of *Jatropha* curcas proteins and application in polyketone-based wood adhesives

Abstract

Jatropha proteins were successfully extracted from the corresponding seeds using the principle of isoelectric precipitation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), elemental analysis and Fourier transform infrared spectroscopy (FTIR) were used to analyze the obtained proteins. The proteins were used at different intakes as a reactive component in polyketone-based wood adhesive formulations. The stability, structure, pot-life and performance of the emulsions as wood adhesive were studied at room temperature by using rheology analysis, confocal fluorescence microscopy and shear strength tests. Emulsions containing proteins were prepared at 45 % solids content, a composition which results in phase separation when using only the reference polyketone-based adhesive. Several factors, such as the particle size (100, 250 μm) of the *Jatropha* proteins and the overall chemical composition of the emulsion, were systematically studied regarding their influence on the product performance. The present study shows that introducing *Jatropha* proteins into the basic recipe of a polyketone-based adhesive constitutes a successful strategy for improving the performance as wood adhesives while at the same time significantly improving the economical attractiveness of these adhesive formulations. All emulsions presented in this work fulfill the requirements for the application of the final product as wood adhesives according to the European EN-314 Standard.

5.1 Introduction

Jatropha curcas L. belongs to the family of Euphorbiaceae. The genus name *Jatropha* is derived from the Greek "iatrós" (doctor) and "trophé" (food) as related to its medical use. It is known in English as "physic nut" or "purging nut", in Dutch as "Purgeernoot" or "Schijtnoot" and in Arabic as "hab el meluk" [1].
Jatropha curcas is a tropical plant/small bush that can grow in low to high rainfall areas (*Figure 5.1*). It can be planted in the form of hedges to reduce erosion and to protect enclosed areas from animals such as goats and cattle since *Jatropha* is considered toxic to humans and animals [2-9]. The tree can reach 3-8 m in height [1,4,5].

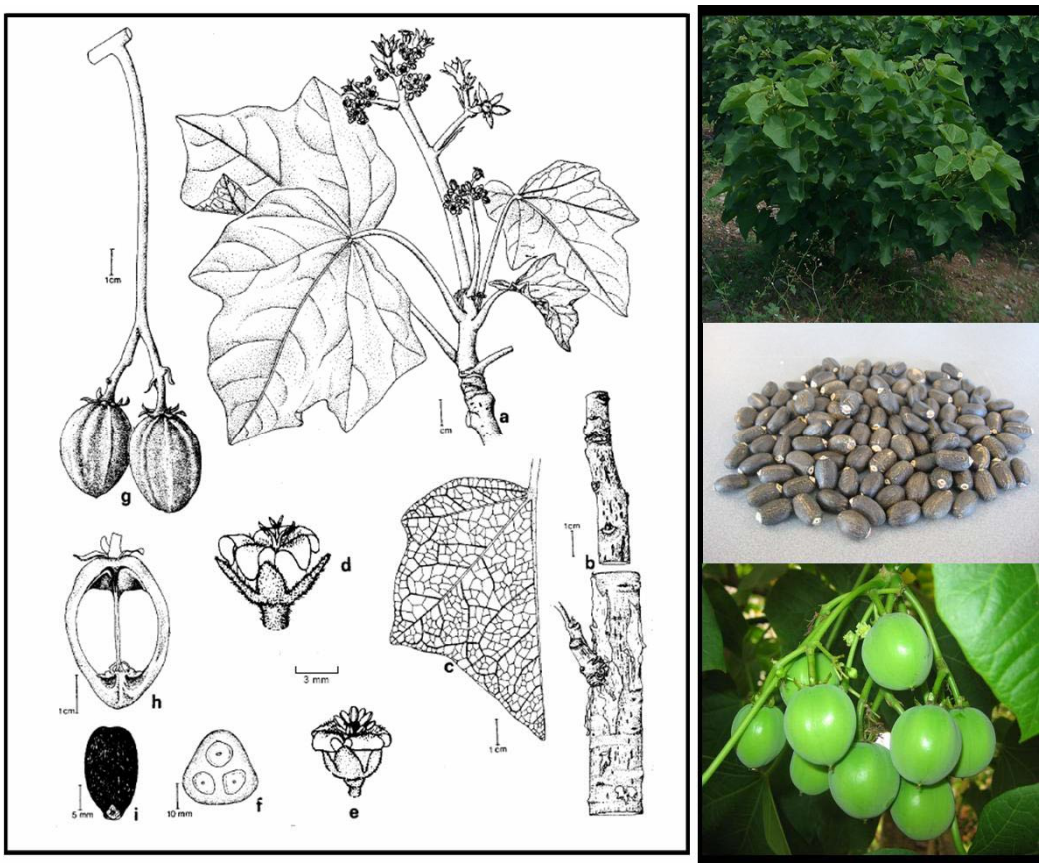

Figure 5.1. Botanical description of important parts of *Jatropha* curcas plant [taken from 3,6] and outlook of the tree and seeds.
a: flowering branch, b: bark, c: leaf, d: pistillate flower, e: staminate flower, f: cross-section of immature fruit, g: fruits, h: longitudinal cut of fruits.

Jatropha is mainly known as a rich source of oil, with an oil content of the kernel reported between 40-66 wt % [5,8,9]. The oil is mainly used as fuel (bio-diesel), in soap manufacturing, and as lubricant in the wood industry [1,2,5,7]. The kernel revealed a protein content of 27-32 wt % while the pressing residue (meal) after oil extraction (fully defatted/degreased meal) has a relatively high protein content around 53-58 wt % [5,8,9].
Several studies showed that both the oil and the meal are toxic to humans and animals. This is due to the presence of phorbol esters and certain proteins (curcines) [1,8,10,11]. Despite this, the relatively high protein content of *Jatropha* meal can be advantageous

since this rich source of protein does not compete with proteins obtained from food crops such as soy and wheat.

Conventional wood adhesives are mainly based on urea-formaldehyde (UF) or phenol-formaldehyde (PF) and known to release formaldehyde to the environment upon production and curing [12-16]. According to the World Health Organization, formaldehyde is suspected to be carcinogen to the human health and harmful to the environment [15,17]. Growing environmental concerns require the development of more environment friendly wood adhesives without formaldehyde emissions [14,15,16]. Several studies have been published on the use of sustainable natural proteins as a feedstock for glue compositions, soy proteins being the most popular example [14,16]. These proteins are abundantly available, renewable, inexpensive, and can be handled with ease and processed at hot and cold press conditions [14,16]. On the other hand, pure soy protein-based adhesives suffer from low bond strengths compared to synthetic adhesives, are sensitive to biological degradation, and show limited water resistance [14-16]. Many chemical modifications have been reported in order to overcome these drawbacks. As an example soy protein was modified with maleic anhydride (MA) and then mixed with polyethylenimine (PEI) [16]. Indeed, the simple modification with MA did not result in a good adhesive and addition of the polyamine seems to be needed to achieve sufficient cross-linking of the matrix upon curing. Despite the addition of PEI the wet shear strength remained below that obtained with phenol formaldehyde (PF) resins. This approach, reaction of soy proteins in their native form or after modification with formaldehyde-free curing agent, is actually quite general as it has also been applied to other biopolymers such as lignin [18]. However, efforts in this direction have been not yet able to overcome the major drawback of natural polymers (in particular soy protein) wood-adhesive: the very poor water resistance.

On the other hand, aqueous polymeric emulsions containing polyketones in combination with chemically modified polyketone (polyamine-mPK30) have been reported as excellent wood adhesives [12,13] with outstanding water-resistance. These adhesives can be easily (one pot synthesis) produced by reacting the polyketone with 1,2-diaminopropane (1,2-DAP) to prepare polyamines. The latter have double functionality, a hydrolytically stable pyrrole ring along the backbone and a reactive pending amino functional group. As such they can be transferred to water soluble cationic compounds by protonation with weak acid (acetic acid). These polyamines act as polymeric surfactants and can thus be used to prepare water-based polyketone emulsions by the addition of a second amount of polyketone.

In our previous study it was shown that replacing part of the active components in the polyketones-based wood adhesive by relatively low molecular weight (~ 7000 Da) soy protein produced an outstanding wood adhesive with excellent performance. Further insight in the role of the soy protein as a co-surfactant and a thickening agent was also reported [19,20]. However, soy proteins are used in the food industry and their use in wood adhesives is less advantageous than using *Jatropha* protein. On the contrary, proteins extracted from *Jatropha* meals constitute in principle a promising renewable protein source that does not compete with the other protein sources in the food chain. Moreover, the relatively high average molecular weight of *Jatropha* proteins (approximately 50 kDa) compared to soy ones could also constitute another profitable point by providing a more stable bond with the wood surface.

The first aim of the present work was to investigate the possibility to extract proteins from the *Jatropha curcas* seeds, using the principle of isoelectric precipitation. The second aim was to apply these proteins in wood adhesive formulations based on the previously

reported polyketone systems. To the best of our knowledge, the use of *Jatropha* proteins in the adhesive formulations has not been reported elsewhere.
In this systematic study the effect of protein particle size and the overall chemical composition on emulsion preparation and product performance was investigated. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) measurement as well as FTIR and elemental analysis were used to characterize the extracted proteins. Moreover rheological analysis was performed to evaluate the stability of the final wood adhesive emulsions. Confocal fluorescence microscopy was used to test the penetration of the glue into the wood. The wood adhesive formulations were tested according to the European EN-314 Standard for wood adhesives [12,13].

5.2 Experimental Part

5.2.1 Materials

Jatropha Curcas seeds were donated by Bandung Institute of Technology (ITB) Indonesia. The seeds were stored in a closed container at 4 ºC prior to analysis. Polyketone with 30 mol % ethene based on the total olefin content (PK30, mass average molecular weight (M_w) of 2670) was synthesized according to a reported procedure [21]. Soy Protein acid Hydrolysate (Soy Pr, Sigma-Aldrich, mass average molecular weight of ~ 7000 Da using MALDI-TOF analysis), Sodium Hydroxide (Merck), Hydrochloric acid fuming (HCL, 37%, Merck), n-Heptane (99+ %, Acros), 1,2-Diaminopropane (1,2-DAP, 99+ %, Acros), Acetic acid (99.5 % pure, Acros), salicylic acid (reagent ACS, Acros), were purchased and used without further purification. Commercial wood maple veneers were purchased from Sikkens Center Groningen (The Netherlands). Double distilled water was used in all experiments.
For the Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE gel electrophoresis) the following chemicals were used: Acrylamide (30 % Acrylamide/Bis, 2.6 % C, Biorad), Tris-HCl (Roche), Sodium dodecyl sulfate (SDS, BOOM, VWR, BDH, PROLABO), tetramethylethylenediamine (TEMED, Biorad), ammonium persulphate (APS, Biorad), glycine (L-Gly, > 98.5 %, Fluka), glycerol (85 %,Merck), β-mercaptoethanol (Biorad), bromophenol blue as a dye (Sigma-Aldrich), Coomassie blue (Serva blue R), ethanol (> 99.9 % , Merck).
Amino acid composition analysis on the *Jatropha* proteins and the soy protein was performed by "Eurosequence B.V Analysis and Synthesis of Protein and DNA" using dedicated HPLC equipment (Aminoquant method) [22], Groningen, the Netherlands.

5.2.2 Preparation of dehulled defatted meal

Jatropha curcas seeds were mechanically crushed using a screw-extruder. Subsequently the seed kernels and hulls were separated manually. Oil was extracted from the kernels using a screw-extruder at 70 ºC. The resulting meal was further defatted by n-heptane extraction in a Soxhlet apparatus using a ratio of 1:4.5 (w/v) for 12 h. The defatted meal was dried in a vacuum oven at 40 ºC until constant weight, then passed through 250 μm-mesh sieve to obtain homogeneous particles and stored at room temperature for further analysis.

5.2.3 Jatropha Curcas Protein Extraction

The principle of isoelectric precipitation was used to obtain the *Jatropha* proteins. A reported methodology for *Jatropha* proteins with slight modifications was used [11]. An

amount of 60 g of defatted and sieved *Jatropha* meal was dispersed in 0.01M NaOH at a ratio of 1:100 (w/v). This process was performed at room temperature. The pH of the dispersion was kept at 11 for 1 h by dosing 0.01M NaOH while the stirring speed was kept at 250 rpm. The insoluble residues were removed by centrifugation at 7000g for 20 min at room temperature. The supernatant was collected and adjusted to pH of 5 using HCl and centrifuged at 7000g for 20 min at room temperature. The resulting white suspension was collected, freeze dried, grinded, sieved to mesh 250 and 100 μm and stored at room temperature for further analysis. A flow sheet for *Jatropha Curcas* protein extraction is shown *Figure 5.2*.

The solubility of the *Jatropha* proteins with particles sieved at 250 and 100 μm was measured at room temperature by dissolving 10 mg/ml *Jatropha* protein in water and stirring for 4 h, the non-soluble parts was filtered over a paper filter grade 4/N – Munktell, and dried in a vacuum oven at 50 ºC until constant weight. A simple mass balance provides the desired solubility values at constant time (4 h).

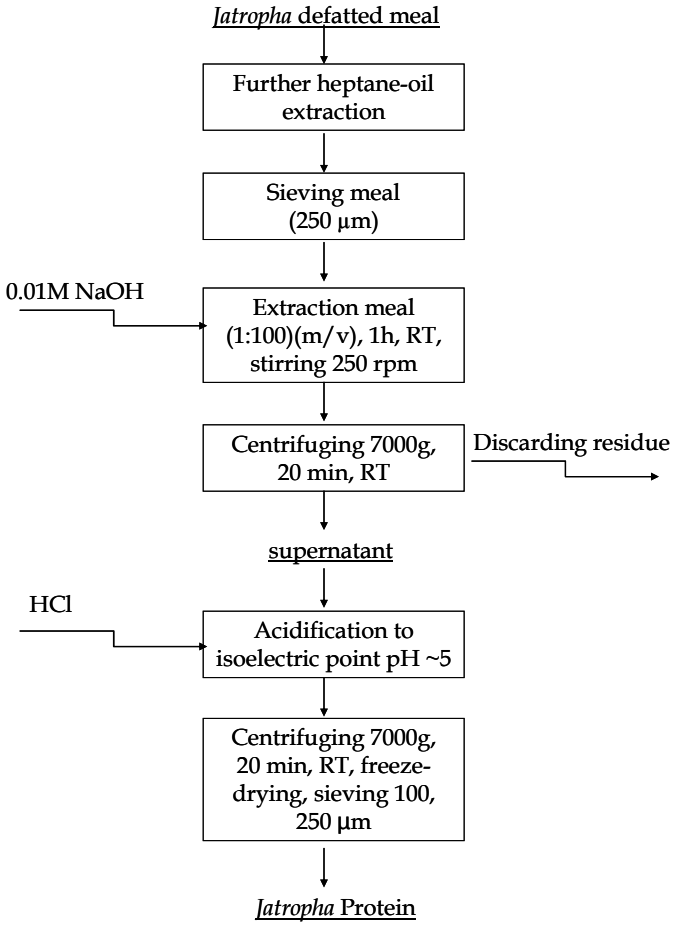

Figure 5.2. Flow sheet for *Jatropha Curcas* L. proteins extraction.

5.2.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE Gel Electrophoresis)

The SDS-PAGE gel electrophoresis was prepared using a slightly modified reported method [23]. The gels containing 12.5 % acrylamide, and stacking gel of 4 % acrylamide, were prepared from a stock solution of 30 % Biorad Acrylamide. The gels were prepared using the following concentrations: 1.5M Tris-HCl (pH 8.8), water and 10 % SDS. The

gels were allowed to polymerize chemically by the addition of tetramethylethylenediamine (TEMED) and 10 % of ammonium persulphate (APS). The gels were prepared between glass plates with slot of 1 mm thickness. Stacking gels of 4 % acrylamide were prepared containing these concentrations: 0.5M Tris-HCl (pH 6.8), water and 10 % SDS. The stacking gels were polymerized in a similar manner as the separation gels. The electrode buffer consists of 30 g Tris, 144.4 g glycine, 10 g SDS and was diluted 10 times prior to use. The samples were prepared using a 'final sample buffer' of the following concentration: 0.5M Tris-HCl (pH 6.8), 10 % SDS, 20 % glycerol, water, β-mercaptoethanol, and 1 % bromophenol blue as a dye. The protein samples were immersed in boiling water for ~5 min and then injected on the gels. Electrophoresis was carried out at 100-200 V until the bromophenol blue marker had reached the bottom of the gels. At the end of this process the gels were stained over night using a solution composed of 0.2 % Coomassie blue (Serva blue R) in 40 % ethanol and 10 % acetic acid. Next, the gels were let to diffusion-distained by repeated washing in 20 % ethanol and 7.5 % acetic acid. The following marker proteins were used: Phophorylase b (97 KDa), Albumin (66 KDa), Ovalbumin (45 KDa), Carbonic anhydrase (30 KDa), Trypsin inhibitor (20.1 KDa), and α-Lactalbumin (14.4 kDa) [24].
The images of the gels were taken using Image Reader LAS-3000, Fujifilm LAS-3000, Intellegent dark box, FujiNon Lens VRF43LMDII.

5.2.5 Emulsion preparation

Polymeric amines were prepared (*Figure 5.3*) by chemical modification of the polyketone by using 1,2-DAP to deliver mPK30. The reaction was carried out according to a well-known procedure [25] in a 250 ml reactor equipped with a reflux condenser, U-type anchor impeller and immersed in an oil bath set at the desired temperature. First the polyketone (40 g, 0.304 mol, calculations based on dicarbonyl units in the PK30 polymer) was charged to the reactor and heated to a temperature of 100 ºC, then 1,2-DAP was added drop-wise (18.02 g, 0.243 mol, based on initial molar ratio between 1,2-DAP and the carbonyl groups in the PK30 of 0.8) in the first 20 minutes of the reaction. The stirring speed was kept constant at 500 rpm. The reactant mixture changed from yellowish to brown and became a solid material upon cooling to room temperature. The prepared polymeric amines were washed several times with double distilled water, filtered and freeze dried. The final product was a light brown powder. The conversion of the carbonyl groups to pyrrole rings was determined by using elemental analysis and found to be around 70 %. These polyamines were converted to water-soluble cationic compounds by protonation with acidic acid solution in double distilled water to match a desired protonation level of 50 %. In the emulsification step (*Figure 5.3*), a second amount of unmodified polyketone (PK30(II)), and *Jatropha* proteins were added. After one hour double distilled water was added to reach the desired solids content (45 wt %). All synthetic steps were performed successively in the same reactor (one-pot process). The resulting emulsions were stored at room temperature in sealed glass jars.

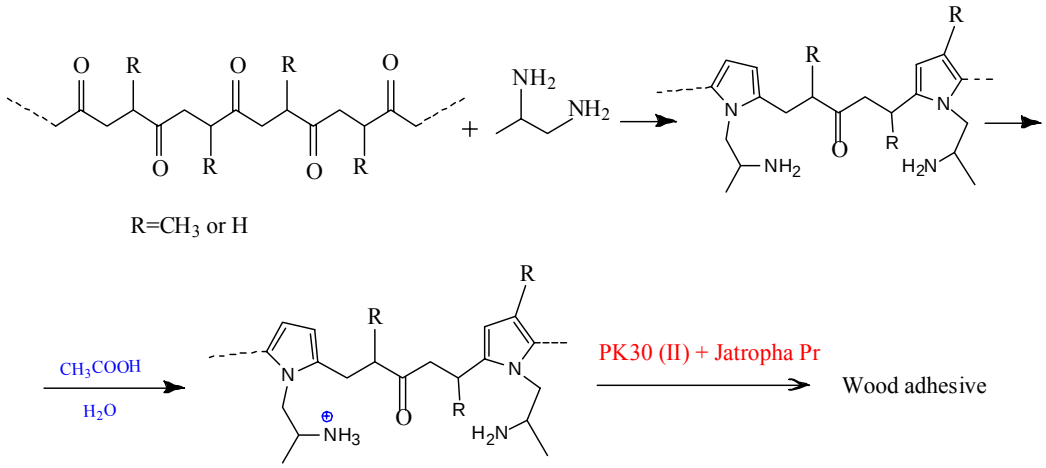

Figure 5.3: Preparation of the *Jatropha* proteins-containing aqueous emulsions.

In Table 5.1 we report the chemical composition of all prepared emulsions on the basis of 1 g polyamine present as surfactant.

Amount of mPK30, (g)	Amount of protein Pr, (g)	Amount of PK30(II), (g)	r_1=PK30(II)/mPK30	r_2=Pr/mPk30
1	0.15	1.05	1.05	0.15
1	0.15	1.35	1.35	0.15
1	0.3	1.2	1.2	0.3
1	0.45	1.05	1.05	0.45
1	0.45	1.35	1.35	0.45

Table 5.1: Chemical composition of prepared emulsions.

The chosen formulation ranges ($1.05 \leq r_1 \leq 1.35$ and $0.15 \leq r_2 \leq 0.45$) and in particular the amount of *Jatropha* proteins used (r_2 values) were fixed on the basis of our previous work on soy protein (by taking into account an increase in viscosity due to the higher average molecular weight of *Jatropha* proteins as compared to soy ones) and of exploratory experiments. The latter clearly demonstrated the occurrence of phase separation (thus no emulsion formation) or very high viscosity values at relatively high protein intakes ($r_2 > 0.45$). One must stress here that optimization of the protein intake does not constitute the aim of the present work as it should be carried out at different total solids contents (only 45 % is presented in this work) and also at different surfactant intakes (i.e. r_1 values outside the range described above) in order to more correctly define the maximum amount of proteins dispersible in the system.

5.2.6 Rheological analysis

The emulsion viscosity (η) was measured at 20 ºC by using an AR 1000 Rheometer (TA Instruments, USA) using an aluminum cone-and plate with a fixture of 2 º cone angle and 40 mm diameter. The apparent viscosity of the samples was measured at a constant shear rate ($\dot{\gamma}$) of 15 s^{-1}. The viscosity-shear rate relationship was established by measurement at different shear rates in the range from 5-60 s^{-1}.

5.2.7 Wood adhesive testing

The maple veneers for the adhesive test were dried at 105 ºC for 10 h to reduce the moisture content to a constant level. A given amount of salicylic acid (0.5 % wt, based on the sum of the second amount of polyketone and *Jatropha* proteins) was used in the emulsion as a curing catalyst. The emulsions were applied at 150 g/m^2 as a single adhesive line onto one side of $25 \times 50 \times 4$ mm^3 maple veneer pieces. The area of the veneer where the glue was exposed was 25×25 mm^2 as reported in a previous study [19]. The specimens were hot-pressed for 5 minutes at 200 ºC under constant pressure of 3 MPa. Ten to thirteen replicates were prepared and tested for each formulation. In line European EN-314 Standard test, the specimens were first immersed in boiling water for 72 h and then cooled in water to room temperature for at least one hour. The shear strength ($\sigma_{strength}$) was measured by using an Instron 4301 machine using 5 KN power sensor with a crossing speed of 2 mm/min.

5.2.8 Confocal Fluorescence Microscopy

The Confocal fluorescence measurements were performed using a Leica (SP2 AOBS) Confocal Microscope with a mercury lamp of 50 W at a 10 times magnification and 400 Hz scan speed. The images were recorded between 501 and 597 nm. The veneer samples were prepared using the procedure as given above for the shear test with the exception of the boiling step in water. Indeed, the veneers, freshly glued, were first sliced and then scanned on the glue line.

5.2.9 Light Microscope

A Zeiss Axiophot light microscope with a CCD DKC-5000 camera was used to make images of the two *Jatropha* proteins sieved at mesh 100 and 250 μm with 20 times magnification.

5.2.10 FTIR analysis

A Perkin-Elmer Spectrum 2000 was used to record the infrared spectra of the proteins. The proteins were placed on the diamond plate and 30 scans were recorded for each sample with a resolution of 4 cm^{-1} over a range of 4000-500 cm^{-1}.

5.2.11 Laser Diffraction (particle size analysis)

Sympatec HELOS (H0503), KA/LA, with dry disperser RODOS, Germany, was used to analyze the particle sizes of the sieved *Jatropha* protein. The dispersion pressure was kept at 3 bars; the laser lamp used was He-Ne at 630 nm, R5 lens: 0.5/4.5….875μm.

5.3 Results and Discussion

The current study focused on the extraction of *Jatropha* proteins from the corresponding *Jatropha Curcas* seeds and on the use of these proteins in polyketone-based wood adhesives.

The proteins were extracted from the meal using an isoelectric precipitation (*Figure 5.2*). In this case the *Jatropha* proteins were solubilized from the corresponding defatted meal (which is brought to a pH of 11 by using 0.01 M NaOH); the proteins were extracted at a pH of 5 by using HCl. The extraction procedure was performed at room temperature

(see experimental part). The yield of the extracted proteins was around 40 wt %. Several analyses have been performed on the extracted proteins such as SDS-PAGE electrophoresis, elemental analysis and FTIR. The SDS-PAGE was used to determine the molecular weight profile for the isolated proteins. The SDS-PAGE patterns of *Jatropha* proteins (*Figure 5.4*) contained three major bands indicating the presence of various types of proteins differing in molecular weight. The molecular weight of the protein subunits were determined by comparing the patterns from *Jatropha* proteins to those from the low molecular weight marker. The top band contained proteins between 30 and 45 KDa, and the other two showed proteins between 20 and 30 KDa.
FTIR analysis [26,27] showed for the extracted *Jatropha* proteins the presence of carbonyl groups (C=O stretching, absorption in the range 1650-1590 cm^{-1}), amino (as in the amide bond) groups (NH stretching above 3000 cm^{-1} and NH bending in the range 1550-1485 cm^{-1}) and hydroxyl groups (OH stretching above 3000 cm^{-1}). Elemental analysis on the *Jatropha* proteins showed an enrichment of protein in the extracted sample: 16.26 wt % of nitrogen (48.43 wt % of carbon) as compared to the original meal which contained only 8.8 wt % nitrogen (40.04 wt % carbon).

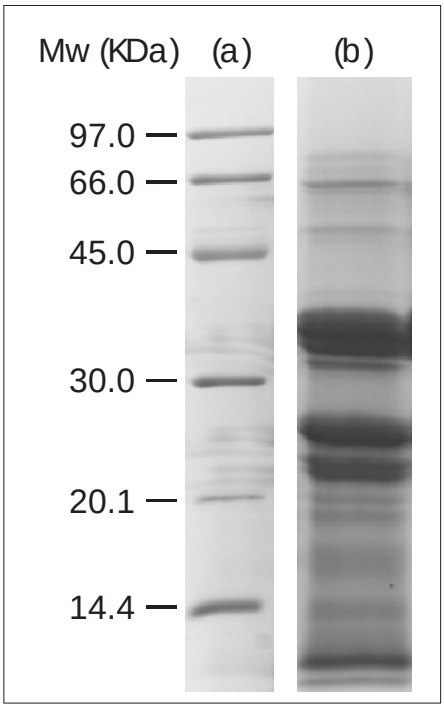

Figure 5.4: SDS-PAGE of the *Jatropha* proteins. (a): low molecular weight marker. (b): *Jatropha* proteins.

The amino acids composition (see experimental part) of the soy (used in our previous work [19,20] and taken here as reference) and *Jatropha* proteins (Table 5.2) revealed similar patterns for the amino acids. The amino acids composition of the *Jatropha* proteins were comparable to those reported in the literature [5,11]. Remarkable for the purpose of this work is the lower percentage of lysine (Lys) in *Jatropha* proteins as compared to soy ones. Indeed, the free amino group of Lys residues can in principle react (via a Paal-Knorr mechanism, *Figure 5.3*), with the carbonyl groups of the unmodified polyketone upon curing of the adhesive, thus providing extra strength to the glue layer between the wood surface. This could constitute a clear disadvantage of *Jatropha* proteins with respect to soy ones in PK-based adhesives. On the other hand, the

relatively higher content of acid groups (see Asx and Glx residues in Table 5.2) could significantly increase the probability of complex formation with the protonated polyamine, a phenomenon already suggested [20] to have a positive influence on the emulsion stability when using soy protein in PK-based wood adhesives. Finally the difference in average molecular weight (*vide supra*) between the two kinds of proteins (higher for *Jatropha*) could also have a positive influence on the adhesion performance as already stated in the aim of the work.
In order to check the influence of the above-mentioned factors on the final product stability and performance, isolated *Jatropha* proteins were used to prepare wood adhesives based on the recipe of polyketone-base adhesives at 45 % solids content. The preparation of this composition was not possible for the basic recipe with only polyketone as it resulted in immediate phase separation [19]. In this study several emulsions were prepared by replacing in the basic polyketone emulsion recipe different amounts of the unmodified polyketone (PK30(II)) with different amounts of *Jatropha* proteins. Several factors, in analogy to what was already established for PK-based adhesives containing soy proteins [19,20], were studied in these emulsions, namely the ratios between the unmodified polyketone PK30(II) to the polyamine mPK30 and the one between the *Jatropha* protein to the polyamine.

Amino acid	abbreviation	Composition (mol%)- *Jatropha* proteins	Composition (mol%)- Soy proteins
Aspartic acid/ Asparagine	*Asx*	9.6	10.0
Glutamic acid/ Glutamine	*Glx*	15.7	19.2
Serine	*Ser*	5.9	2.1
Histidine	*His*	2.3	1.7
Glycine	*Gly*	8.9	7.5
Threonine	*Thr*	4.3	2.3
Alanine	*Ala*	7.7	10.9
Arginine	*Arg*	9.7	5.8
Tyrosine	*Tyr*	2.2	2.3
Valine	*Val*	6.5	5.9
Methionine	*Met*	1.5	1.3
Phenylalanine	*Phe*	4.3	4.2
Isoleucine	*Ile*	5.2	5.2
Leucine	*Leu*	8.0	7.9
Lysine	*Lys*	2.9	6.4
Proline	*Pro*	5.2	7.4

Table 5.2: Amino acid composition of *Jatropha* proteins and Soy proteins.

The only extra factor included in this study, but not reported for soy protein, is the average particle size (inclusive distribution) of the solid proteins. Indeed, as *Jatropha* proteins, in contrast to soy ones are only partially soluble in water we assumed in first instance that the solubilization kinetics (dependent on particle size) might be comparable to the emulsification (dispersion) one. In other words, if solubilization and dispersion of the protein are characterized by similar timescales, there could be an influence of solubilization on the dispersion of the protein between the dispersed and continuous phase. Since emulsions are metastable structures, equilibrium (i.e. phase separation) is usually not attained at relatively short times and a hypothetical difference in the partition of the protein chains (as due to different particle size) might result in relevant differences in the emulsion structure and performance. In order to check this hypothesis, two different particle sizes (obtained by sieving at 100 μm and 250 μm) of the *Jatropha*

proteins were used to prepare emulsions at 20 wt % *Jatropha* proteins intake. The structure and performance of the prepared emulsions were analyzed. Rheology measurements on the emulsions showed that the viscosity is a function of the particle size of the *Jatropha* proteins (*Figure 5.5*). Emulsions with particle size sieved at 250 μm showed much higher viscosity than those of particle size sieved at 100 μm. In line is the visual observation that the emulsion obtained from the sample with particle size sieved at 100 μm showed a more homogeneous structure/texture than the one obtained from the sample sieved at 250 μm. Indeed, the latter showed the formation of sediment of larger particles (average size in the order of around 1 mm) in the first five days. After this the emulsion remained stable for 1 month storage time, while the one prepared with smaller particles remained perfectly stable for all 30 days.

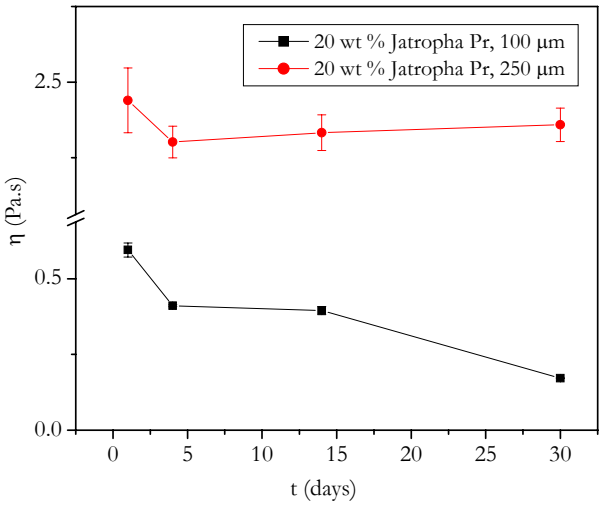

Figure 5.5: Effect of particle size of *Jatropha* proteins on the viscosity of the emulsions.

Although the reason for such behavior is yet to be fully clarified, one must notice here that a similar behavior, i.e. a dependency of the emulsion structure and stability on the particle size before emulsification, has been observed also for different systems [28-31]. In order to get a better insight into this effect, we performed a detailed analysis of the average particle size (distribution) for the two kinds of protein particles.

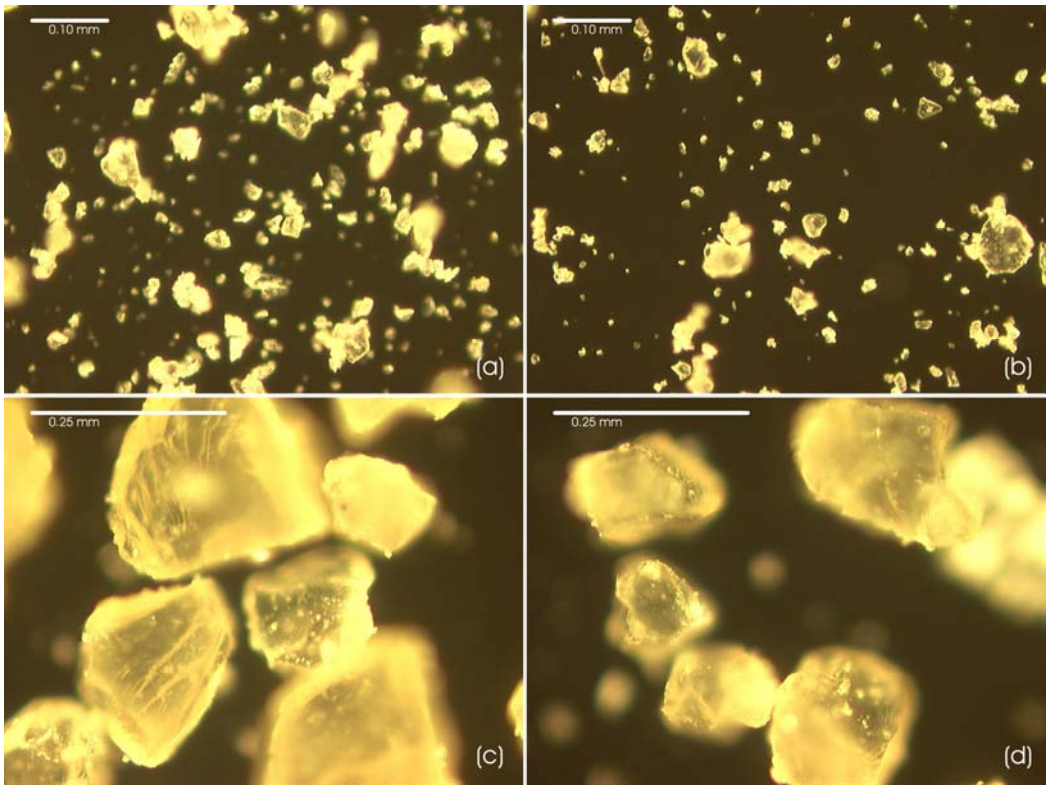

Figure 5.6: Light microscope picture of *Jatropha* proteins at X 20 magnification: (a and b): Particle size sieved at 100 μm. (c and d): Particle size sieved at 250 μm.

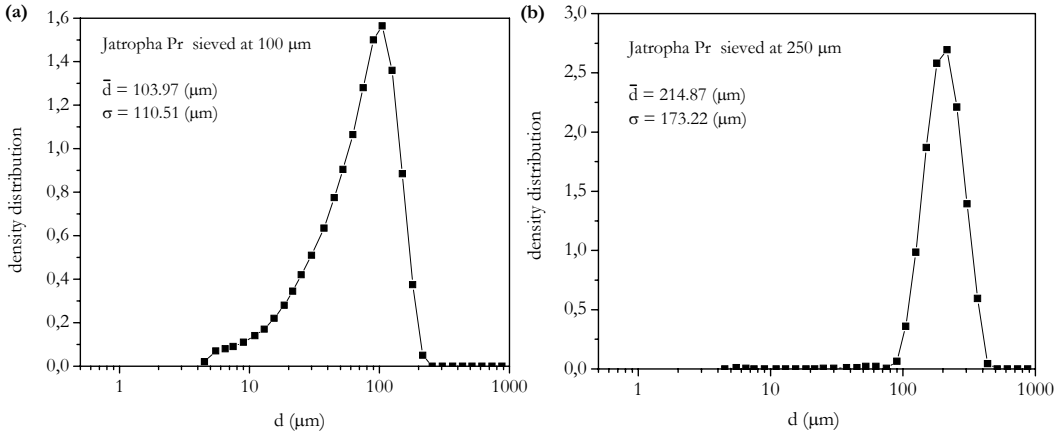

Figure 5.7: Particle size distribution of *Jatropha* Pr, $\bar{d}$ is the average particle size of the *Jatropha* Pr (μm) and σ is the width of the density distribution of the particle size at half the height. a): Particle size sieved at 100 μm. (b): Particle size sieved at 250 μm.

It seems from the microscope pictures (*Figure 5.6)*, that the particles sieved at 250 μm are less homogeneous in size than those sieved at 100 μm (the latter form aggregates clearly seen in the microscopic pictures). The laser diffraction for particle size analysis (*Figure 5.7*) showed an average particle size ($\bar{d}$) of 103.97 μm and 214.87 μm of the samples sieved at 100 μm and 250 μm respectively. The width (□) of the particle size distribution of the sample sieved at 250 μm (*Figure 5.7-(a)*) is higher than the sample sieved at 100 μm

(*Figure 5.7-(b)*), thus confirming the microscope observation concerning the homogeneity of the two kinds of particles. Moreover, the measured solubility at room temperature at constant time scale for the two particle sizes *Jatropha* Pr is slightly different: the 100 μm particles displayed a solubility of about 26 % while 250 μm ones of about 20 %. Such effect of the particles size on the solubility/dispersability, in agreement with previous observations also on different systems [19,28-31], might have an effect on the kinetics of the solubility and therefore on the emulsification process (*vide supra*) and is currently under investigation.

The consequences of this difference of the emulsions structure on the final product performance as wood adhesives were investigated by immersing the corresponding wood panels (see experimental part on wood-adhesive test) in boiling water for 72 h, then cooling them to room temperature and finally by performing shear strength measurements. All samples (i.e. after the shear test) contained fibers from the opposite veneer. This indicates that the glue is stronger than the wood. The shear strengths measurements resulted in all cases in higher values than those required by the European standards. However, the samples prepared with particle size sieved at 250 μm showed a slightly lower shear strength value (3.2 ± 0.4 MPa) with respect to the ones sieved at 100 μm (3.5 ± 1.1 MPa). This effect, although debatable considering the experimental error, is expected based on viscosity differences. The 250 μm sieved particle size sample has a higher viscosity which makes the application/spreading on the wood surface less favorable, thus resulting in slower/more difficult spreading and penetration into the wood surface. This hypothesis is clearly confirmed by the confocal fluorescence microscopy pictures and the correspondingly generated 3-D views (*Figure 5.8*). It implies less penetration of the glue into the bulk of the wood in the case of samples of particle size sieved at 250 μm.

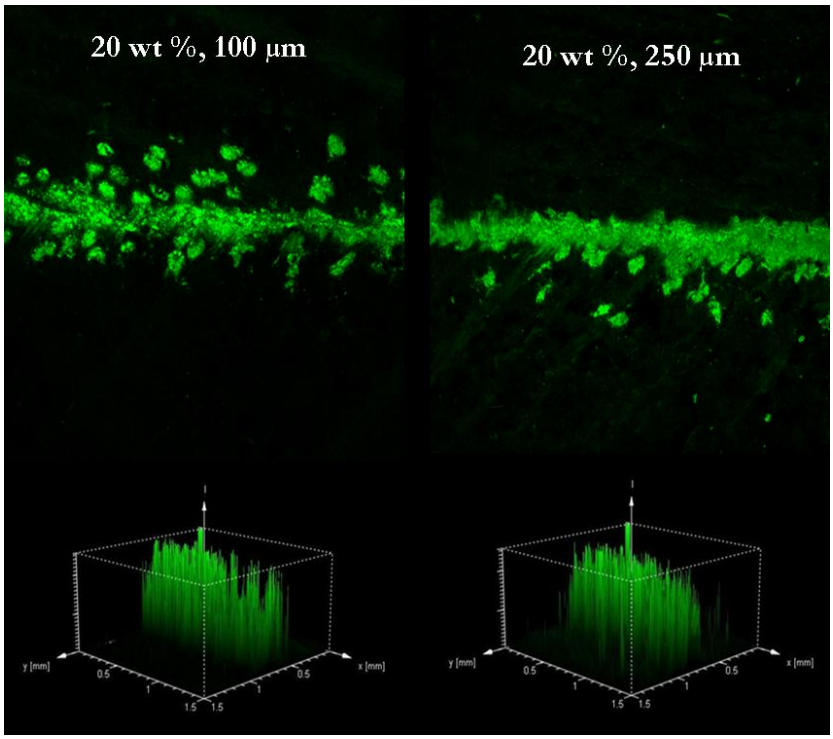

Figure 5.8: Confocal fluorescence microscopy of 20 wt % intake of *Jatropha* proteins based emulsions at two particle sizes. Left: particle size of *Jatropha* proteins sieved at 100 μm. Right: particle size of *Jatropha* proteins sieved at 250 μm.

Based on the favorable results for the viscosity analysis and the shear strength tests it was decided to use the *Jatropha* proteins with particle size sieved at 100 μm to prepare a new set of emulsions. In a first series (rows 2-4 in Table 5.1), emulsions were prepared by substituting/adding different amounts of *Jatropha* proteins (10, 20 and 30 wt % with respect to the unmodified polyketone, PK30(II)) by keeping the ratio (*r)* between the *Jatropha* proteins and the unmodified polyketone to the polyamine (PA) constant and equal to 1.5 wt/wt.

$$r = \frac{\text{amount of proteins} + \text{amount of unmodified polyketone}}{\text{amount of polyamine}} = 1.5 \qquad (5.1)$$

This experimental plan finds its conceptual origin on the initial assumption that the *Jatropha* proteins simply act as a dispersed phase in the emulsion (i.e. displaying only a slight surface activity due to the limited solubility), in a comparable role (i.e. dispersed phase) to the one of the unmodified polyketone. The stability and performance of the emulsions were studied as a function of storage time at 45 wt % solids content using rheological and shear strength analysis respectively.

The viscosity of the emulsions showed a clear dependence on the amount of proteins present in the overall formulation (*Figure 5.9*). It can be seen that higher amounts of proteins in the system resulted in an increase in the viscosity. This confirms our previous hypothesis (from our study on soy proteins system), and other published data, that the proteins play a role as thickening agent and give extra stabilization by generating repulsive interactions of both steric and electrostatic nature in the dispersion [19,20,32,33]. Such differences in the rheological behavior could result in different performance of the emulsions as wood adhesives. However, the shear strength value of the sample with 10 wt % proteins intake was 3.0 ± 0.6 MPa while 20 and 30 wt % proteins intakes resulted in 3.5 ± 1.1 MPa and 3.4 ± 0.4 MPa respectively. Despite the positive fact (from a purely applicative point of view) that all samples showed higher shear strength values than that required by the European EN-314 Standards, no significant differences (see averages and the corresponding standard deviations) are observed as function of the proteins content. This is surprising if one takes into account (*Figure 5.9*) the clear differences in the rheological properties.

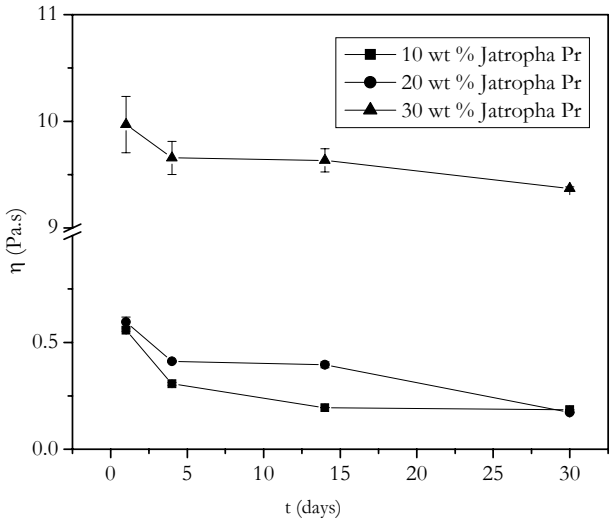

Figure 5.9: Effect of 10, 20 and 30 wt % *Jatropha* proteins intake on the viscosity of the emulsions.

However, such invariance of the product performance as function of the proteins intake is further confirmed by the confocal fluorescence microscopy pictures (*Figure 5.10*), which can be used to gain information about the penetration of the glue in the bulk of the wood [34]. All samples showed good broad glue lines (top of *Figure 5.10*) while the corresponding 3-D views (bottom of *Figure 5.10*) did not highlight any significant differences regarding the penetration depth in the bulk of the wood panel.

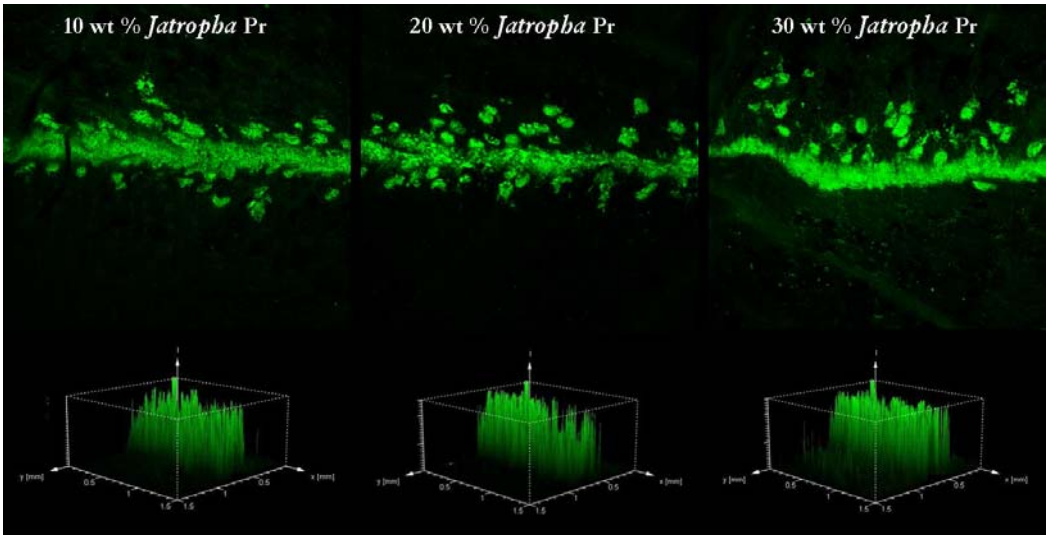

Figure 5.10: Confocal fluorescence microscopy of 10, 20 and 30 wt % *Jatropha* proteins intake emulsion.

The explanation of such contrasting results (i.e. viscosity vs. wood adhesive tests and fluorescence images) is probably related to the interplay between two different factors affecting the wood adhesive tests. The viscosity of the final product might have a negative influence on the performance (*vide supra*), i.e. higher viscosity results in less prominent penetration, but constitute only one of the factors affecting the product

performance. Relatively higher proteins intake (thus higher viscosity) results also in an increased polarity of the final emulsion, thus promoting the adhesive penetration on the (polar) wood surface. A substantial balance of these two contrasting factors might explain the observed discrepancy.
The more prominent polarity of the polypeptide chains (with respect to the unmodified polyketone) has consequences also from a conceptual point of view. The data discussed above all refer to emulsions in which the proteins are basically considered as filler for the system, i.e. a chemical that, together with the virgin polyketone, must be emulsified in the presence of the polymeric surfactant (polyamine). However, this is not completely correct since proteins, as a consequence of their polar character, might also act as co-surfactant for the system [19]. This means that emulsions containing different amounts of proteins could display different behavior because of the different amount of surface-active species (polyamine and proteins) present in the formulation.
To test these hypotheses, we prepared a second series of emulsions (rows 1-2 and 3-4 Table 5.1) for which two ratios were *independently* changed: the one between the unmodified polyketone to the polyamine (r_1), and the one between the *Jatropha* proteins to the polyamine (r_2). Rheology measurements showed a clear dependence of the emulsions viscosity on the two ratios, r_1 and r_2 (*Figure 5.11*).

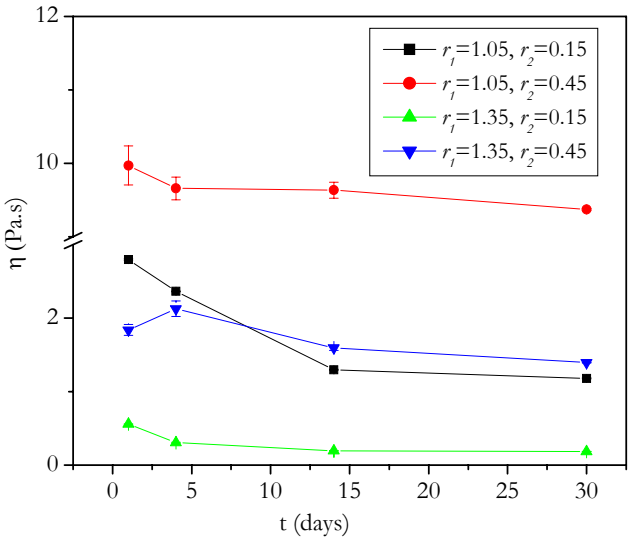

Figure 5.11: Viscosity of emulsion as a function of storage time. (a): at a fixed r_1=1.05 as function of r_2. (b): at a fixed r_1=*1.35* as a function of r_2.

At constant r_1 value (*Figure 5.11*), the viscosity increased by increasing the protein intake (r_2) in the emulsions. This result confirmed our previous findings that the proteins act as a thickening agent, and gives extra stabilization to the dispersions. The intermolecular interactions increase due to the unfolding in the protein molecules, which results in increasing the viscosity, the major forces that promote such interactions being probably of electrostatic nature [31].
At constant r_2 value (*Figure 5.11*), the emulsion viscosity decreases as function of the unmodified polyketone amount (r_1). This in agreement with our previous finding that a relative decrease in the surfactant amount (in this case corresponding to higher r_1 values) generally results in lower emulsion viscosity [19].
Shear strengths results showed that all the prepared emulsions passed the test with higher shear strengths than required by the European EN-314 Standard test (Table 5.3).

r_1	r_2	$\sigma_{strength}$, MPa
1.05	0.15	3.4 ± 0.7
1.05	0.45	3.4 ± 0.4
1.35	0.15	3.0 ± 0.6
1.35	0.45	3.3 ± 0.6
1.5	0	2.8 ± 0.2 [(a)]

Table 5.3: Shear strength of samples at different r_1 and r_2 values after 1 day.
[(a)] Reference sample with no protein added to the system at 50 % total solids content.

From a purely applicative point of view one must stress here the fact that all emulsions performed significantly better than the reference sample (last row in Table 5.3) containing only PK. In this respect the use of *Jatropha* proteins constitute a significant improvement of the product quality and performance. However, from a scientific point of view, also in this case (*vide supra*), comparable shear strength values are obtained when comparing the different samples either at a fixed r_1 or r_2 value. The only exception is represented by the sample with r_1=1.35 and r_2=0.15, which showed a very slight decrease of □□ with respect to the others. Such deviation from the general trend is not surprising if one takes into account that this sample has a relatively high PK amount (r_1=1.35) and a relatively low proteins intake (r_2=0.15), both factors having a negative influence on the shear strength values. Apart from this exception, the rule seems to indicate an independence of the performance as wood adhesives on the overall composition. This might reflect a similar structure of the corresponding emulsions or simply the relatively low accuracy of the measurements (see corresponding experimental errors in Table 5.2). The latter hypothesis seems to be more reasonable given the difference in the emulsion viscosity (and thus probably in the emulsion structure) and the confocal fluorescence images (in contrast to what was observed in the first series). Indeed, at fixed r_1 values, (*Figure 5.12*), it can be seen that a thicker glue line and more noticeable penetration in the wood were noticed at higher proteins intake. Such effect is probably due (see above) to a change in the overall polarity of the formulation: the presence of peptide chains, which have higher affinity for the wood surface than the apolar polyketone ones, is probably responsible for this effect. Furthermore, at fixed r_2 values, a more relevant wood penetration and a thicker glue line were noticed from the samples at higher r_1 (more virgin polyketone is present).

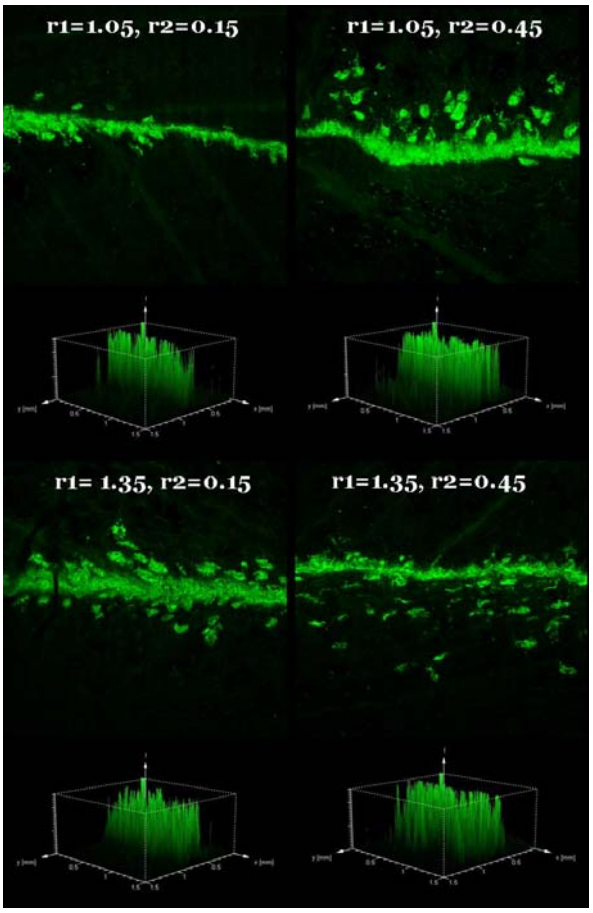

Figure 5.12: Confocal fluorescence spectroscopy images of emulsions as a function of both ratios r_1 and r_2.

The obtained results indicate only slight differences, if any, in the adhesive structure and performance as a function of the overall composition. This is only in partial agreement with our results on a similar system based on soy proteins [19,20]. The viscosity of the emulsion with soy proteins showed much higher values than those with the *Jatropha* ones (*Figure 5.13*). Moreover, shear strength analysis showed that the *Jatropha* protein-containing emulsions gave a significantly better shear strength (3.5 ± 1.0 MPa) than the one of the soy proteins emulsions (2.6 ± 0.6 MPa). A simple statistical analysis [35] (t-test) clearly confirms (p-value of 0.013) the significance of the observed difference. This is expected since lower viscosity adhesives are easier to apply on the wood surfaces, which might mean better flow properties and hence a deeper penetration in the bulk of the wood (*vide supra*). These differences in the structure and performance between the two protein-based emulsions might be due to the differences in their chemical or dimensional structure. It is well known that proteins experience conformational changes when adsorbed at interfaces to minimize the number of unfavorable interactions and maximize the favorable ones in the new environment [33]. The time needed for such changes to take place and the molecular flexibility by folding and unfolding of the proteins in the mixture differs from one protein to the other [36].

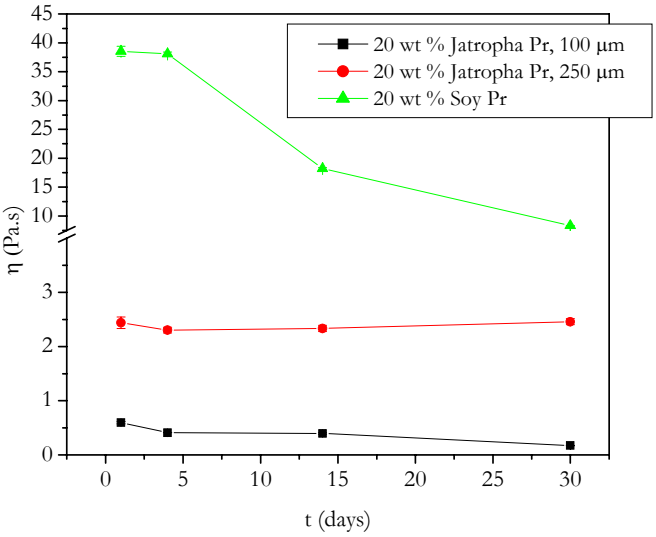

Figure 5.13: Viscosity of soy protein emulsions and *Jatropha* protein emulsions at 20 wt % intake as a function of storage time.

Furthermore, soy proteins are completely soluble in water [19,20] whereas *Jatropha* proteins are only partially soluble as mentioned before. This may be related to the different average molecular weight (higher for *Jatropha* proteins-see experimental part) as well as amino acid composition (Table 5.1). For the soluble soy proteins, we already proposed [20] a possible mechanism, by which these components affect the emulsion stability and structure, based on the hypothesis that the corresponding polypeptide chains are absorbed on the surface of the polyketone particles. A similar study would be required also for the *Jatropha*-based emulsions in order to elucidate and possibly explain the observed differences. From an application point of view, the observed results clearly point out the "versatility" of these wood adhesives formulations, whose behavior can be finely tuned also as function of the kind of proteins added to the system.
Finally, from a product technology point of view, the obtained results clearly indicate the necessity of a more comprehensive toolbox for the (molecular) design of new wood adhesives that are partially based on natural products. The current study of the emulsion viscosity and wood penetration should be coupled with a more fundamental and accurate investigation of the protein physical properties (e.g. polarity, solubility and surface activity) to explain, finely tune and possibly predict the final product performance as wood adhesive.

5.4 Conclusions

The choice for *Jatropha* protein to be used in PK-based emulsions is mainly due to the fact that the corresponding meal is a rich source of protein, the extraction of the protein is a low cost process, and most importantly this rich source of protein does not compete with proteins obtained from other food crops such as soy and wheat.
A successful extraction procedure to obtain proteins from the *Jatropha* seeds using the principle of isoelectric precipitation was applied. Several analytical techniques, such as SDS-PAGE electrophoresis, elemental analysis and FTIR spectroscopy, clearly confirmed isolation of the proteins from the corresponding meal. The obtained *Jatropha* proteins were used in the preparation of aqueous protein-containing polymeric emulsions

based on chemically modified thermosetting polyketones in a one-pot process. The amount of proteins added to the basic formulation has a clear influence on the emulsion stability and viscosity (around 10 Pa.s) as well as on the corresponding penetration depth in wood veneers. Only minor changes were observed in the wood performance test, probably as a result of the relatively low experimental accuracy (i.e. relatively larger experimental error). Nevertheless, the latter clearly demonstrated that all prepared emulsions qualify as wood adhesives according to the European EN-314 Standard with shear strengths values of more than 3 MPa which is higher than the minimum requirement value of 1 MPa. In this respect, the use of *Jatropha* proteins (only partially soluble in water as compared to the fully soluble soy ones) results in better performance as wood adhesive with respect to soy. This suggests a different stabilization mechanism for the corresponding emulsion (yet to be studied), but clearly widens up the number of possibilities for different kinds of natural products to be added to the basic polyketone emulsion in order to prepare new wood adhesives that fulfill the requirement set by the European standard.

5.5 Abbreviations

PK30(II): second amount of polyketone 30 mol % ethene content based on the total olefin content, unmodified polyketone
mPK30: modified polyketone 30 mol % ethene with 1,2-diaminopropane, (polyamine)
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
FTIR: Fourier transform infrared spectroscopy
h: hour(s)
η : viscosity (Pa.s)
$\dot{\gamma}$: shear rate (s^{-1})
d: particles size of the *Jatropha* Pr (μm)
$\bar{d}$: The average particle size of the *Jatropha* Pr (μm)
σ: width of the density distribution of the particle size at half the height
r: is the ratio between *Jatropha* proteins and unmodified polyketone to the polyamine
r_1: is the ratio between unmodified polyketone to the polyamine
r_2: is the ratio between *Jatropha* proteins to the polyamine
$\sigma_{1\ strength}$: shear strength after 1 day (MPa)
Pr: protein

5.6 References

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Chapter 6: Prospects of proteins and other natural materials in the production of polyketone-based wood adhesives

6.1 The use of proteins in polyketone/amine formulations

The current instability of the oil market, the continuous concern of long term supply of the oil resources and the strict environmental regulations regarding the emissions from formaldehyde-based compounds, result in an urgent need for more environmentally friendly wood adhesives [1-4]. Natural materials such as proteins are promising replacements for synthetic resins [1-3]. Some disadvantages of using protein-based adhesives are their low durability, water and mould growth resistance, and a short pot life [1-3]. R&D activities in this area therefore heavily focus on improvement of these product properties to become competitive with existing synthetic resins.

This work describes the preparation of aqueous polymeric emulsions based on both modified polyketones and natural products (Soy and *Jatropha* proteins) and their use as wood adhesives. The overall process consists in the chemical modification of thermosetting alternating polyketones with di-amines by the Paal-Knorr reaction [5]. The obtained polymeric amines act as a surfactant in the emulsions and can be used to disperse the virgin polyketone in water. The emulsions were qualified as wood adhesives according to the European Standard for wood adhesive testing EN-314 [5]. However, the drawback of these formulations is two-fold: limited flexibility in terms of the final formulation (below 50 % solids content these emulsions are not stable and display phase separation over time). A high anticipated price and limited availability of the polyketones.

By introducing natural proteins (Soy or *Jatropha*) in the basic PK-recipe, both issues can be at least partially tackled. The research on amine/polyketone/protein formulations presented in this work show that it is possible to substantially improve the stability of the emulsions (thus significantly expanding the range of available solids contents). In addition, the polyketone intake (and therefore the overall emulsion cost) may be reduced considerably (up to 30 wt % of both proteins can be used in the final formulation instead of the original PK) without any negative effect on the product performance and even displaying a better penetration into the wood matrix (chapters 3, 4 and 5). Furthermore, the inherent drawback associated with the use of natural proteins, namely a low water resistance of the corresponding adhesives formulations, does not constitute a problem for the presented formulations. Finally, from an environmental point of view, the advantage of these protein based polyketone adhesives lies in the properties of both the proteins and polyketones. Proteins are considered a renewable source, abundantly available, inexpensive, and easy to handle at hot and cold press conditions, while polyketones are biodegradable [1-3,6]. This clearly confers a "green" character to these new products.

6.2 Alternatives for soy and Jatropha proteins

This research provided a proof of concept for the use of proteins in PK/amine formulations in order to reduce the price of the adhesive and to improve flexibility (e.g. in terms of lower solids contents) without compromising the product properties and its stability. Two protein sources were selected (soy and *Jatropha*) and successfully applied. Further investigations with a broader range of biopolymers containing amine groups

would be highly desirable. Indeed, if amino groups are present on the natural polymer at relatively high concentration, a reaction with the PK upon curing (possibly via the Paal-Knorr mechanism) is expected to improve the network hardness, thus leading to better adhesive performance.
In this respect chitosan might represent a suitable choice for the natural polymer. Chitosan is a polysaccharide containing primary amine groups (*Figure 6.1*), derived from the alkaline deacetylation of chitin by treatment with potassium hydroxide or sodium hydroxide [7-14]. Chitosan is unique among other biopolymers due to the presence of primary amino groups along the backbone at relatively high concentration (one group per monomeric unit). Protonation of these groups renders the polymer partially or totally soluble in acidic media [7,10,11,13].
In the last 25 years, chitosan has received attention as a functional biopolymer because of its high biocompatibility, ecological safety and low toxicity in a wide range of applications. Examples are its use in the formulation of nutrition, medicine, cosmetics, fabric and textile conditioners, and in water treatment agents. In addition to this, chitosan has also shown to be an attractive catalyst support [8,9,11-13,15,16].

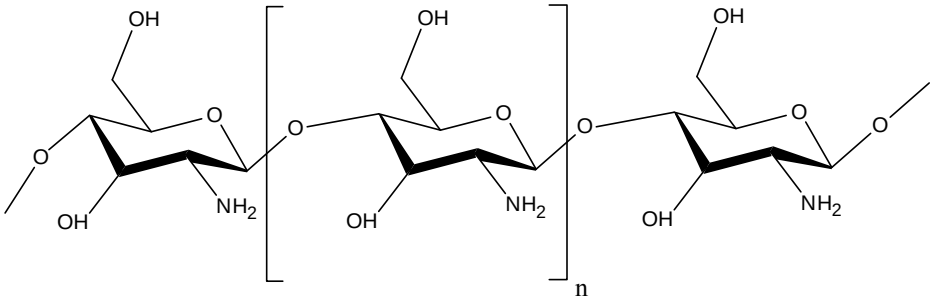

Figure 6.1: Basic structure of chitosan.

The presence of the amino groups makes chitosan a very attractive ingredient in the PK-based wood-adhesive formulation. The primary amino groups (Chapter 2) are expected to react easily with the polyketone. This hypothesis is tested by using chitosan (Sigma-Aldrich, Mw of ~50 KDa) for the preparation of water based emulsions for wood adhesive applications. In the standard polyketone based recipe, 10 wt % of chitosan was used to replace the unmodified polyketone according to the procedure reported in Chapters 3 and 5. The stability and performance of the emulsion was studied as a function of time using rheology measurement and shear strength analysis respectively. Emulsions with 20 wt % *Jatropha* proteins were compared with the 10 wt % chitosan containing emulsion. This difference in natural product intake and average molecular weight (100-150 kDa for chitosan, 30-45 kDa for *Jatropha* proteins), was appropriate because of the similar initial viscosity of both emulsions (*Figure 6.2*), which allows a better comparison from a purely applicative point of view.

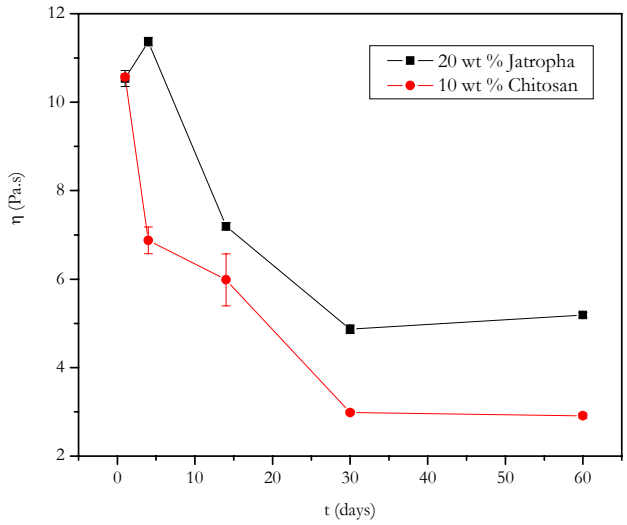

Figure 6.2: Effect of storage time on viscosity of chitosan emulsions at 50 wt % solids content.

Both emulsions were stable for at least 60 days storage time, thus confirming chitosan as a valid alternative to *Jatropha* proteins in terms of pot-life. Moreover, a better product performance for wood adhesives applications was obtained from the chitosan-based emulsion as compared to *Jatropha*. Shear strength values of 3.0 ± 0.4 MPa and 3.1 ± 0.6 MPa after 1 and 60 days respectively compare favorably to the values for the *Jatropha*-based product (2.4 ± 0.7 MPa and 2.8 ± 0.7 MPa after 1 and 60 days respectively). A statistical comparison [17] (t-test) between these values indicate (p-values of 0.020 and 0.356 after 1 and 60 days respectively) a clear difference for the freshly prepared emulsions between chitosan and *Jatropha*, the former resulting in a better performance. Despite the relatively low intake of chitosan (only 10 wt % with respect to the unmodified polyketone), this clearly suggests a difference in the corresponding structure of the emulsions. The differences in performance are clearly attenuated after 60 days storage time. Future studies are needed to understand the reasons for this observation. Nevertheless, the chitosan-based emulsion passed the European Standard EN-314 shear strength test with significantly higher values than required (1 MPa).
Independently of the nature of the used biomaterial (either chitosan or natural proteins), formulations with PK result in a clear advantage with respect to the PK-based adhesives. Indeed since it is possible to obtain similar performance at relatively lower solids content (Table 6.1), the amounts of mPK30 and PK30(II) are significantly lower.

Sample	Total solids content (wt %)	mPK30:[PK30(II)+ biomaterial]	Amount of PK30(II), (g)	Amount of mPK30, (g)	Amount of biomaterial, (g)	$\sigma_{strength}$, MPa
Reference sample	45	1:0.9	21.3	23.7	0.0	3.1 ± 0.7
Soy Pr	45	1:1.2	18.4	20.5	6.1	2.7 ± 0.5
Jatropha Pr	45	1:1.5	18.9	18.0	8.1	3.4 ± 0.4
Chitosan	50	1:1.5	27.0	20.0	3.0	3.0 ± 0.4

Table 6.1: Composition and properties of some reference and biomaterial-containing emulsions. Calculations based on 100 g emulsion.

6.3 Further improvements by process optimization

Proteins are complex 3-D polymers made from the combination of 20 different amino acids with different polar and non-polar properties linked via peptide bonds [18]. Despite the many possible combinations, proteins are generally considered as natural polymeric surfactants, because they contain both hydrophobic and hydrophilic amino acid residues. However, such surface activity is clearly dependent on the properties of the proteins themselves such as size (molecular weight), charge, structure, degree of denaturation, stability, and the balance between polar, non-polar and charged amino acids [18]. This aspect also plays a role in this research and clear differences in product performance were observed for adhesive emulsions of soy and *Jatropha* proteins (Chapter 5). The pH of the emulsion is expected to be an important factor in both the stabilization and formulation of the emulsions in particular with respect to the amount of the mPK30. As a consequence, the stability of the protein containing emulsions at different neutralization degrees was explored. The standard PK-adhesive formulation, comprising only water, unmodified PK and modified polyketones (polyamine) as surfactant, is only stable at acid conditions (pH $\leq$ 6) due to the necessity for the amino groups on the polyamine to be at least partially protonated in order to display a surfactant-like activity (*Figure 2.8*, Chapter 2). On the other hand, the presence of proteins might significantly change this situation due to the presence of chemical groups in the system (e.g. carboxylic acids) that can be neutralized at pH values higher than 7. We thus examined a PK-based glue (*Figure 6.3-(a)*) and compared it with one containing Jatropha proteins (*Figure 6.3-(b)*, 10 wt % protein, 45 wt % solids content) by systematically changing the emulsions pH (by the addition of either 1 M NaOH or 1 M HCl).

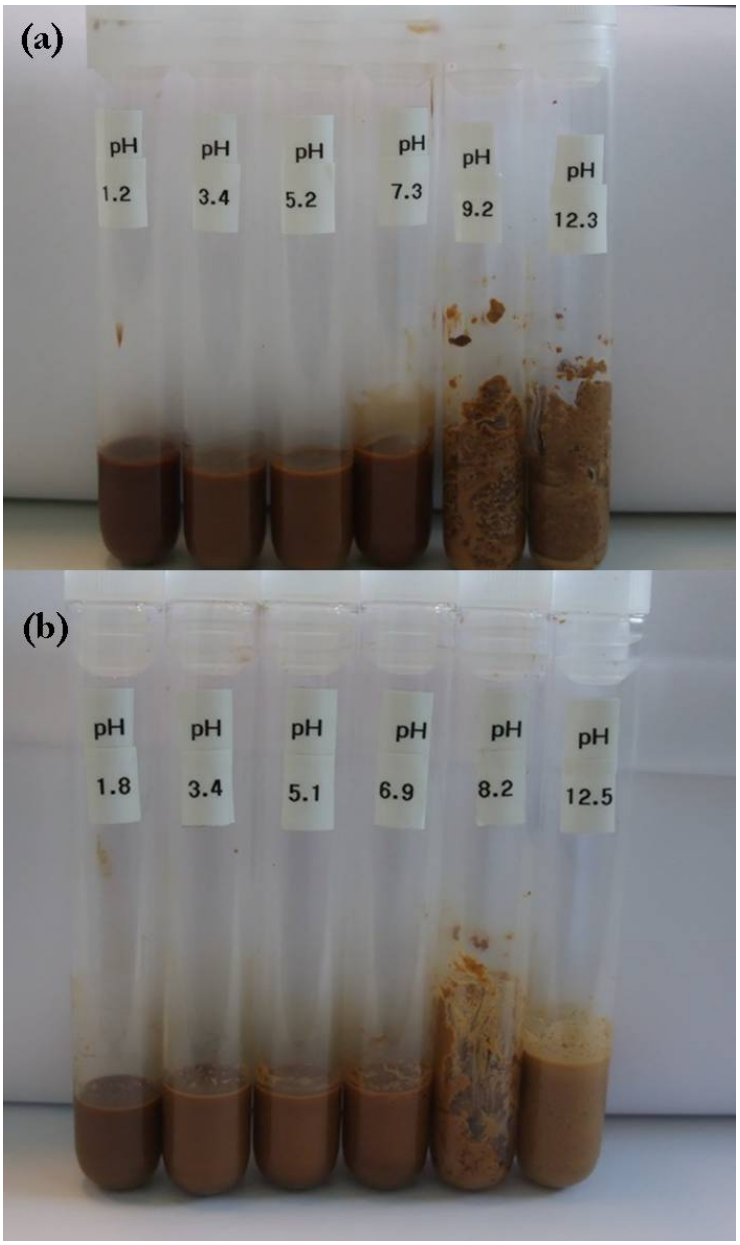

Figure 6.3: Neutralization effect on (a) PK-standard emulsion and (b) the *Jatropha* containing emulsions

At relatively low pH values (≤8), the PK-emulsion (*Figure 6.3-(a)*) are stable as expected and no phase separation is observed. By increasing the pH to ~ 8, an immediate separation and precipitation can be observed for the PK-adhesive. This happens in a range of pH where the protonated polyamines are not stable and precipitate (*Figure 2.8*, Chapter 2). Further increase in the pH values does not have any significant effect on the emulsion, which thus remains instable and gives phase separation. Up to pH=8 the same kind of behavior is observed for the emulsion containing Jatropha proteins (*Figure 6.3-(b)*). However, further increase in the pH to a value ~12 or higher, caused the emulsion to switch back to stable behavior, a phenomena not observable in the absence of proteins and probably due to partial ionization of the carboxylic acid groups on the peptide chains. In this respect one might speculate a

synergic mechanism consisting in a dynamic change of roles: at high pH the polyamine is simply de-activated (in terms of surface behavior) and might become factually a part of the dispersed phase while the proteins assume now the role of surfactant in the system. From a purely scientific point of view such hypothesis would suggest a very dynamic character of the interface chemical composition, a concept worth studying in future work. From an applicative point of view, one might start noticing how such observation reflects/mirrors completely our results (Chapter 2) on the surface activity of the products from the model reactions between amino acids and polyketone. In this sense, also the reaction product of PK with Lys, displaying the presence of both amino (ionized at relatively low pH values) and carboxylic acid groups (ionized at relatively high pH values) along the backbone, could display a similar behavior to the one of the proteins [19]. The use of such surfactant alone in the formulation or together with proteins could help even further in the stabilization of the emulsion (and thus probably in its performance as adhesive) in a broader range of pH values.

6.4 Conclusions

The work presented here has shown a proof of principle for the production of a new 100 % formaldehyde free environmentally friendly wood adhesive based on polyketones/amines and natural proteins. The use of the protein is expected to lead to a considerable reduction in the cost-price of the formulation. From a scientific point of view, a better understanding of the role of proteins in the emulsions has been achieved in this work. However, further studies are needed in order to get deeper insight of the interface dynamics (for example as function of pH) and of the adhesion mechanism on the wood surface. From a product performance point of view, the use of other natural polymers (chitosan) as well as of new amphoteric surfactants could help in achieving even better adhesion to the wood surface as well as a better emulsion stability as function of pH. Both factors could significantly widen the range of applications available for these chemical products.

6.5 Abbreviations

R&D: research and development
PK: polyketone
Mw: molecular weight
PK30(II): second amount of polyketone 30 mol % ethene content, unmodified polyketone
mPK30: modified polyketone 30 mol % ethene with 1,2-diaminopropane, Polyamine
Pr: protein
wt %: weight percentage
$\sigma_{strength}$: shear strength (MPa)
t: time (days)
η : viscosity (Pa.s)

6.6 References

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Summary

The current market for wood adhesives is predominantly based on formaldehyde-containing resins. The emissions of formaldehyde in the preparation, curing process or during the application is known to be harmful to the environment and suspected to be carcinogenic according to the World Health Organization. Besides, the current economic situation and the trend towards the use of greener bio-based chemicals show that there is a need for developing environmental-friendly wood adhesives.
Biorelated materials are abundantly available and may serve as an interesting starting material for the preparation of inexpensive, biodegradable, and renewable (components in) wood adhesive formulations. Well known examples are the use of proteins, tannins and lignins, carbohydrates, casein etc... Unfortunately, natural sources-based wood adhesives suffer some major drawbacks such as relatively low strength, low water resistance, and sensitivity to biological attack. Many modifications and blends have been proposed (***Chapter 1***) to improve the properties of such adhesives and for instance to make them water resistant.
A potentially very interesting novel wood adhesive formulation comprises natural proteins and polyketones. The presence of reactive electrophilic carbonyl groups on the polyketone chains as well as nucleophilic groups (e.g. $-NH_2$) on the protein suggest the possibility of chemical reaction between the components, for example upon curing of the two components between two wood surfaces. ***Chapter 2*** describes model compound reactions between polyketones and several different amino acids via the Paal-Knorr reaction. The reaction results generally in the formation of pyrrole rings along the polyketone backbone with pendant carboxylic acid groups as a second functional group along the backbone, *Figure A*. As a result, a new class of polymeric surfactants was obtained.

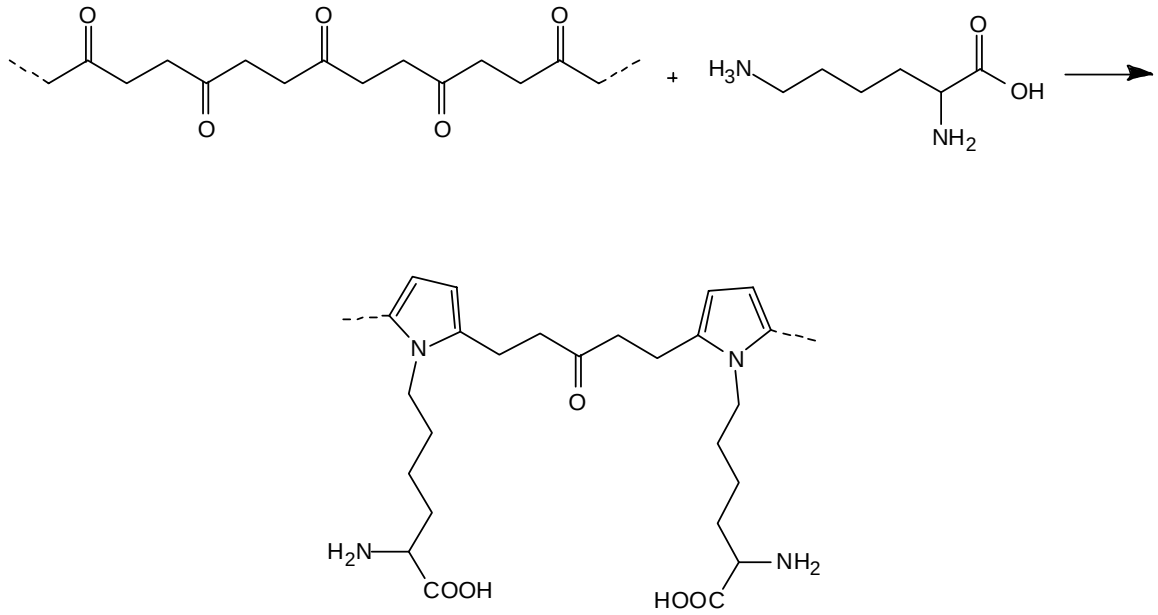


Figure A: The proposed Paal-Knorr reaction between polyketones and amino acids (Lys and PK with only ethylene as olefin component are taken as examples).

Initially, the reaction between 2,5-hexanedione (a model compound for the alternating polyketones) and different amino acids was performed to characterize the final products and

to gain insights in the rate of the reactions. The final conversion of the amino acids is strongly dependent on the steric hindrance of the amino groups, a result also observed when using polyketones. The reaction between polyketones and amino acids was carried out using two synthetic approaches: a conventional in a glass reactor with convective heating and a novel approach (to the best of our knowledge never reported before) using microwave irradiation. The latter method gave higher conversion rates and a higher final yield. The reaction products from both synthetic ways (conventional and microwave) showed interesting surfactant properties in aqueous solutions. Relevant properties of the compounds were determined by drop tensiometry, fluorescence spectroscopy, optical activity measurements and X-ray photoemission spectroscopy (XPS). The products show interesting chiral properties, which opens a wide number of possibilities for the application of these new polymeric surfactants for chiral separation processes.

Recently novel wood adhesives based on polyketones were invented and explored in detail. The adhesives are formaldehyde-free and environmental-friendly. Furthermore, they may be prepared conveniently in a one pot process (*Figure B2-(I)*). The wood adhesives are prepared by reacting a polyketone with a di-amine leading to the formation of poly-pyrroles with pendant amine groups. The resulting polymeric amines act as surfactants and were used to disperse a larger amount of virgin polyketone in water. The emulsions were tested as wood adhesive and shown to meet the European Standard for wood adhesive testing (EN-314). A drawback of the current system is the limited flexibility in terms of the final formulation (e.g. the emulsion is stable only at a narrow range of total solids contents). In this respect the addition of natural polymers (i.e. proteins) to the formulation could be advantageous and lead to lower costs of the wood adhesive (proteins are a relatively cheap feedstock) and enhanced stability and performance of the wood adhesive. In ***Chapter 3***, the introduction of soy proteins to the basic recipe of the polyketones based adhesives (*Figure B2-(II)*) is described.

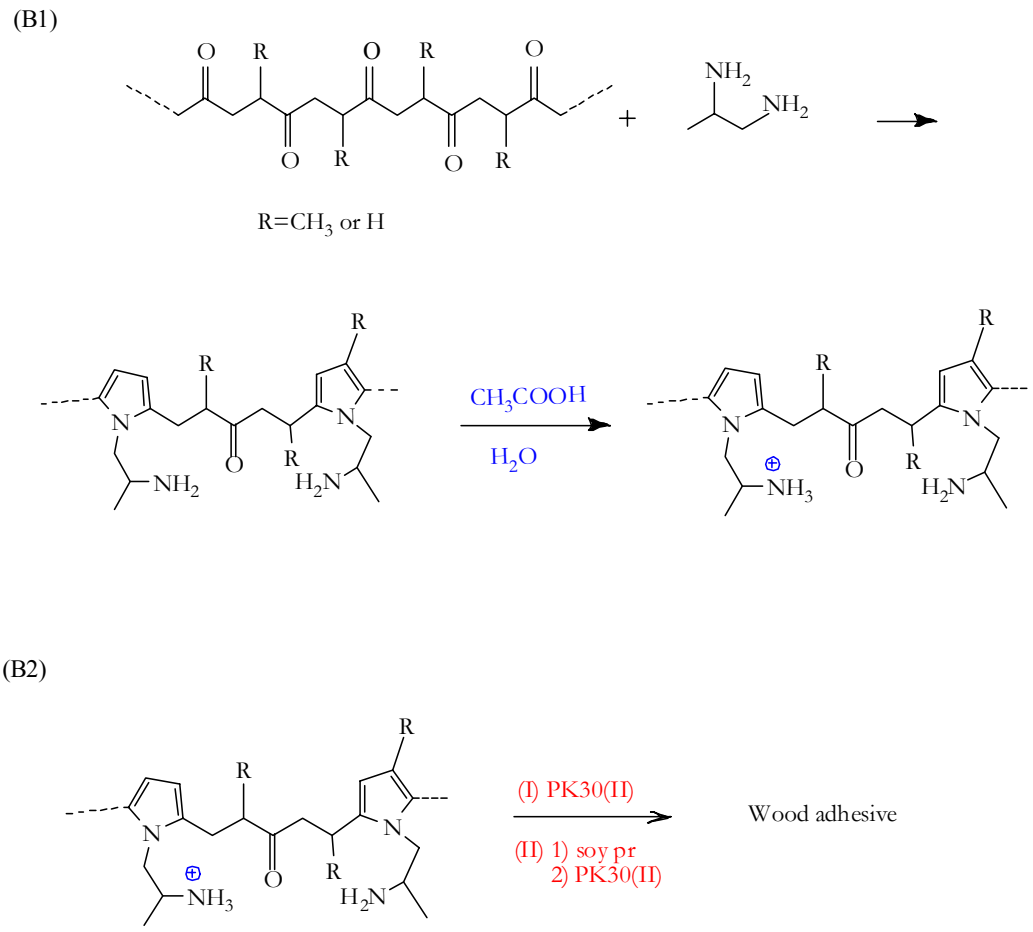


Figure B: Preparation of the protein-containing aqueous emulsions. B1: emulsification step (polyamine preparation). B2: preparation of the wood adhesive formulation

Various emulsions were prepared with different solids contents (40, 45, and 50 wt %) and addition protocols. Emulsions with 45 and 40 % solids content could be prepared, a composition which results in phase separation when using polyketones only. The stability and the structure of the emulsions were studied as a function of time at room temperature. Emulsions with an average particle size less than 1 μm and a viscosity less than 1 Pa.s at 40-45 wt % solids content could be prepared and were stable for more than 6 months. All protein-containing emulsions passed the European standard (EN-314) wood test with higher shear strength than required (1 MPa). However, the viscosity of the protein-containing emulsions was slightly higher than the reference sample (no protein present). It was shown that the presence of up to 40 wt % proteins (with respect to the amount of virgin polyketone) in the adhesive formulation results in a slight increase in the average particle size as well as the viscosity, meanwhile the performance as wood adhesive is not affected significantly. Confocal fluorescence microscopy showed the presence of broader glue lines (improved glue penetration in the wood) in the presence of proteins compared to the reference polyketone-based adhesive. The main advantage of the use of soy proteins in the polyketone-base formulation is retention of the performance as a wood adhesive but at relatively lower solids contents (45 vs. 50 % with respect to adhesives prepared only with polyketones).

Chapter 4 describes a systematic study on the effect of the ratio of the soy protein/ unmodified polyketone intake and the chemically modified polyketone (i.e. the polyamine surfactant) intake at a specified solids content of 45 % wt. The stability, shelf life and the performance of the formulations as wood adhesives were studied at room temperature. The results were modeled using multiple linear regression ($R^2 \geq 0.961$) to predict the shear strength after 1 day of emulsion preparation. It was shown that all emulsions were qualified as wood adhesives according to the European Standard for wood adhesive test EN-314. Furthermore, an insight in the role of the soy protein on the stability and performance of the emulsions was obtained by surface tension measurements and the use of model (low molecular weight) compounds. These measurements clearly showed complex formation between the proteins and the protonated polyamine. Complex formation is responsible for a higher surface activity of the surfactant and therefore for the stabilization of the emulsion. In conclusion, it appears that the proteins act as co-surfactants and thickening agent at the same time.

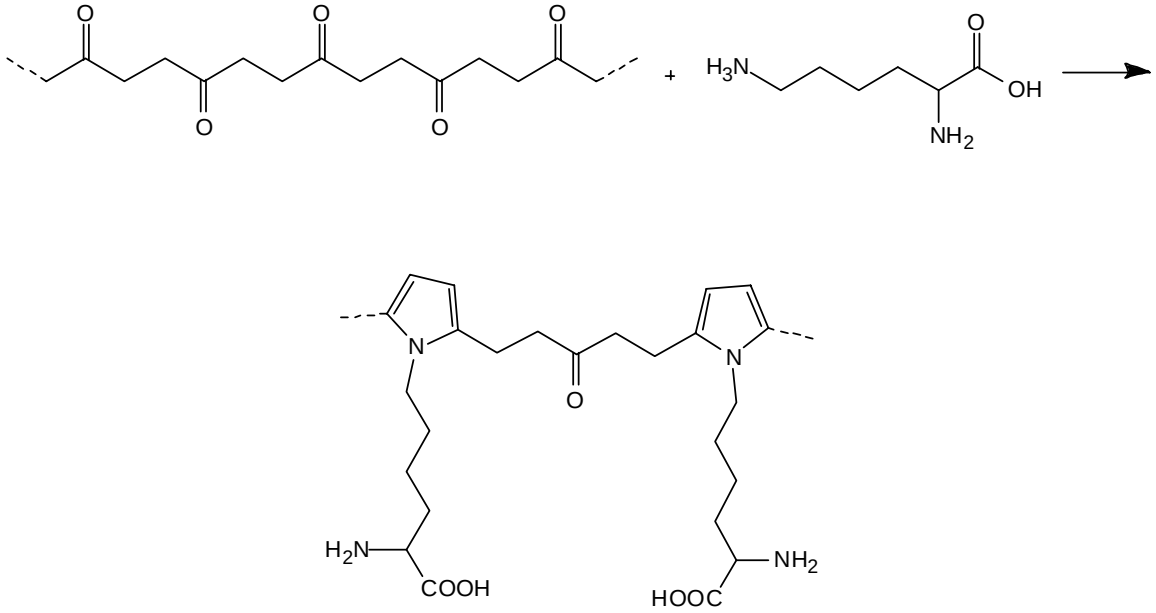
To determine the effect of the protein source in the formulation on the emulsion stability and performance, other proteins were studies as well. *Jatropha curcas* L. proteins were successfully extracted from the seeds using isoelectric precipitation ***(Chapter 5)***. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), elemental analysis and Fourier transform infrared spectroscopy (FTIR) were used to analyze the proteins. The proteins were used to prepare aqueous protein-containing polymeric emulsions from chemically modified thermosetting polyketones in a one-pot process. Several factors that could influence the product performance were studied, such as the particle size of the *Jatropha* proteins and the overall chemical composition of the emulsion. Moreover, the stability, structure, pot-life and performance of the emulsions were studied by rheological analysis, confocal fluorescence microscopy and shear strength tests. It was found that the protein content in the basic formulation has a clear effect on the emulsion stability and viscosity as well as on the corresponding penetration depth in the wood. However, the effects on typical wood adhesive properties (e.g. shear strength) were only marginal, possibly due to the relatively low accuracy of the measurements.

Finally, a chapter about the prospects of proteins and other natural biorelated materials in the production of polyketone based wood adhesives (***Chapter 6***) is presented which discusses the potential of the findings in this thesis to prepare polyketone-based wood adhesives containing natural biopolymers like chitosan. In addition, technological and scientific challenges for future research on the formulations to become techno-economically attractive are highlighted.

Samenvatting (Dutch summary)

Er is een grote behoefte aan milieuvriendelijke en duurzame houtlijmen. De huidige markt voor houtlijmen is voornamelijk gebaseerd op formaldehyde bevattende formuleringen. De emissies van formaldehyde gedurende de bereiding en tijdens het gebruik staan bekend als schadelijk voor het milieu en hebben volgens de Wereld Gezondheid Organisatie een verdacht carcinogene werking. Groene grondstoffen uit bijvoorbeeld biomassa kunnen dienen als interessante startmateriaal voor de bereiding van goedkope, bio-degradeerbare en hernieuwbare (componenten) in houtlijm formuleringen. Bekende voorbeelden zijn het gebruik van eiwitten, tannines, lignine, koolhydraten en caseïne. Helaas hebben deze groene houtlijm formuleringen een aantal nadelen zoals een relatief lage sterkte, lage waterresistentie en gevoeligheid voor biologische afbraak. Een breed scala aan modificaties in de basismaterialen en formuleringen zijn voorgesteld om de eigenschappen van de lijmen te verbeteren (***Hoofdstuk 1***).

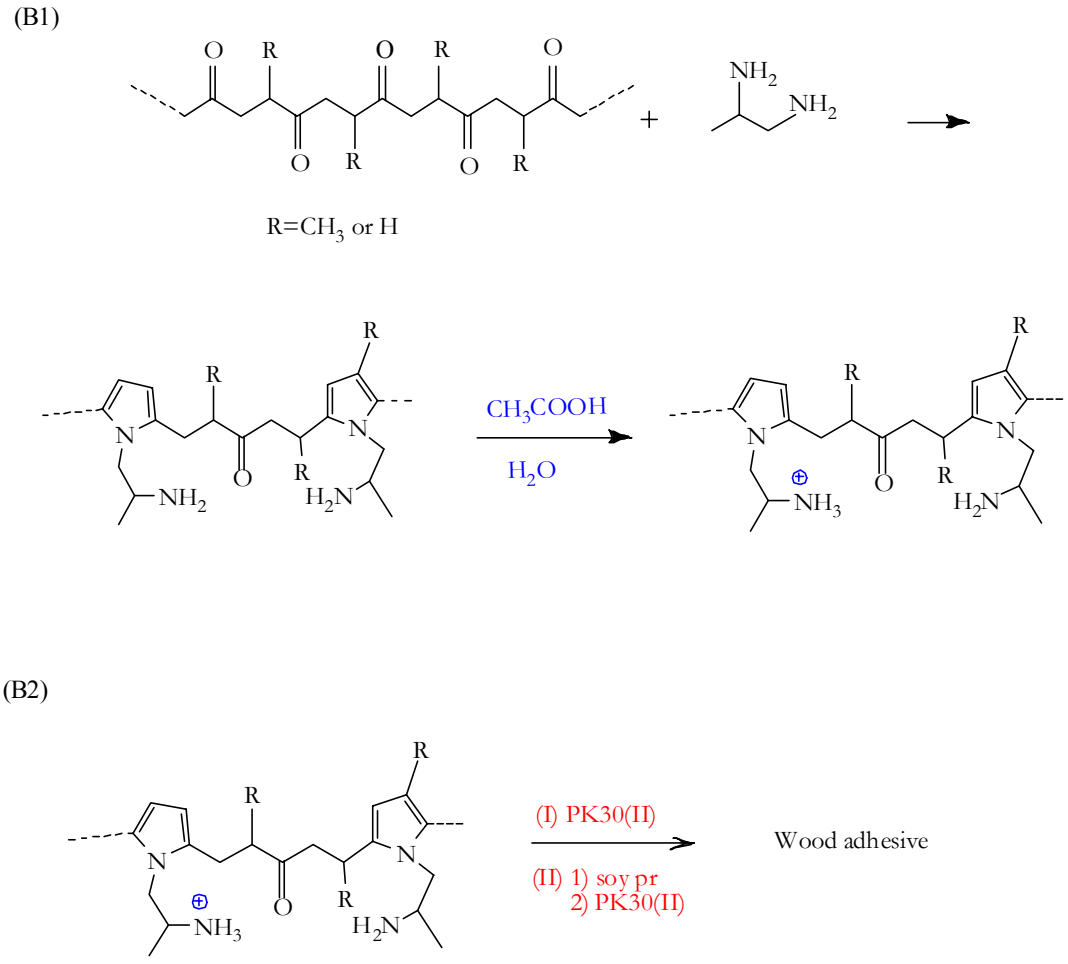
Dit proefschrift beschrijft onderzoek naar een nieuwe houtlijm formulering op basis van natuurlijke eiwitten en polyketonen. De reactieve electrofiele carbonyl groepen in de polyketon ketens en de nucleofiele groepen (bv. $-NH_2$) van de eiwitten geven de mogelijkheid voor een cross-linking reactie. ***Hoofdstuk 2*** beschrijft model reacties van polyketonen met verschillende aminozuren via de Paal-Knorr reactie. Deze reactie resulteert in de vorming van pyrrole ringen in de polymeer keten en zure zijgroepen, *Figuur A*.



Figuur A: De Paal-Knorr reactie van polyketonen met aminozuren (Lys en PK met alleen ethylene als olefinische bouwsteen zijn genomen als voorbeeld).

Initieel is de reactie van 2,5-hexadione (een model component voor alternerende polyketonen) met verschillende aminozuren bestudeerd om de uiteindelijke producten goed te kunnen karakteriseren en inzicht te krijgen in de snelheid van de reacties. De uiteindelijke conversie van de aminozuren en de reactie kinetiek zijn sterk afhankelijk van de sterische hindering van de amino groepen. De reacties van polyketonen met aminozuren zijn uitgevoerd met convectieve verwarming en microwave straling. De laatste methode gaf een hogere conversie en opbrengst. Relevante eigenschappen van de producten zijn bepaald met

druppel tensiometrie, fluorisentie microscopie, optische activiteit metingen en foto-emissie spectroscopie (XPS). De reactie producten laten interessante oppervlakte actieve eigenschappen zien in waterige oplossingen. De producten zijn chiraal en hebben een breed scala aan mogelijkheden om toegepast te worden voor chirale scheidingsprocessen.
Recent zijn nieuwe houtlijmen gebaseerd op polyketonen gerapporteerd en in detail bestudeerd. De lijmen zijn formaldehyde vrij en milieuvriendelijk. Bovendien kunnen ze gemaakt worden in een 1 pot synthese proces (*Figuur B*). De houtlijmen worden gemaakt door een polyketon met een di-amine te laten reageren wat leidt tot de vorming van poly-pyrrolen met amine zijgroepen. Deze polymere amines gedragen zich als surfactanten en zijn gebruikt om een grotere hoeveelheid ongemodificeerde polyketon te dispergeren in water. De resulterende emulsies zijn getest als houtlijm en voldoen aan de Europese Standaard (EN-314). Een nadeel van het huidige systeem is de gelimiteerde flexibiliteit met betrekking tot de formulering (bv. de emulsie is stabiel in een beperkt totale vaste stof gehalte bereik).
In verder onderzoek is geprobeerd om een deel van de polyamines te vervangen door goedkope amines in de vorm van natuurlijke eiwitten. In ***Hoofdstuk 3*** worden soja eiwitten geïntroduceerd in het basis recept van de polyketon gebaseerde lijmen (*Figuur B).*



Figuur B: Bereiding van eiwit bevattende waterige emulsies. B1: polyamine bereiding. *B2*: bereiding van de houtlijm formulering.

Een groot aantal emulsies met verschillende vaste stof gehaltes (40, 45 en 50 % massa) zijn bereid via een aantal procedures. Er zijn stabiele emulsies met 40 en 45 % vaste stof gehaltes gemaakt. Zonder eiwittoevoeging resulteert dit in alle gevallen in fase scheiding. De stabiliteit en structuur van de emulsies zijn bestudeerd als functie van de tijd. Emulsies met een gemiddelde deeltjes grootte van 1 μm, een viscositeit minder dan 1 Pa.s bij 40-45 % massa vaste stof gehalte zijn stabiel gedurende 6 maanden bij kamertemperatuur. De eiwit houdende emulsies voldoen aan de Europese Standaard (EN-314) test voor houtlijmen met een hogere afschuifspanningen dan vereist (1 MPa). Echter de viscositeit van de eiwit bevattende emulsies is ietwat hoger dan het referentie monster zonder eiwit. De aanwezigheid van tot wel 40 massa % aan eiwitten (gerelateerd aan de hoeveelheid polyketon) in de formuleringen resulteert in een kleine toename in de gemiddelde deeltjesgrootte en de viscositeit, terwijl de eigenschappen van de houtlijm niet significant verminderden. Confocale fluorescentie microscopie laat de aanwezigheid zien van brede lijmlijnen in het gelijmde hout bij gebruik van eiwitten in vergelijking met de referentie polyketon gebaseerde lijm en dit duidt op een verbeterde penetratie van de houtlijm componenten in het hout.

Hoofdstuk 4 beschrijft een systematische studie naar het effect van variabele verhoudingen van het soja-eiwit en de andere componenten in de formulering bij een vaste stof gehalte van 45 massa %. De stabiliteit en de eigenschappen van de resulterende houtlijmen zijn bestudeerd bij kamertemperatuur. De afschuifspanning van de emulsie is ook bepaald en gemodelleerd ($R^2 \geq 0.961$). Alle formuleringen voldoen aan de Europese standaard voor houtlijmen (EN-314). Verder inzicht in de rol van de soja eiwitten op de stabiliteit en andere emulsie eigenschappen is verkregen met oppervlaktespanning metingen en het gebruik van laag moleculaire model componenten. Deze metingen laten complex vorming zien tussen de soja eiwitten en de geprotoneerde polyamines. Deze complex vorming is verantwoordelijk voor een hogere oppervlakte activiteit van de surfactant en leidt tot een hogere stabiliteit van de emulsies. Concluderend kan gezegd worden dat de eiwitten zich gedragen als co-surfactant en verdikkingsmiddel.

Naast soya eiwitten zijn ook andere natuurlijke eiwitten getest zoals eiwitten uit de zaden van de *Jathropha curcas* L. heester (***Hoofdstuk 5***). Electrophorese (SDS-PAGE), element analyse en FTIR zijn gebruikt om de eiwitten te karakteriseren. De stabiliteit en structuur van de eiwit houdende emulsies zijn bestudeerd met een aantal technieken (reologie analyse, confocale fluorescentie microscopie en afschuifspanning testen). Het eiwit gehalte van de formulering heeft een duidelijk effect op de stabiliteit en viscositeit van de emulsies en ook op de eigenschappen van de resulterende houtlijmen (bv. penetratie diepte in het hout).

Hoofdstuk 6 handelt over het gebruik van polyketon-gebaseerde houtlijmen met natuurlijke amine bevattende bio-polymeren zoals chitosan in de formulering. In dit hoofdstuk worden ook de technologische en wetenschappelijke uitdagingen gegeven voor verder onderzoek aan dit type formuleringen die moeten leiden tot groene, verbeterde en goedkopere houtlijm formuleringen.

ملخص (Arabic summary):

تعتمد المواد اللاصقة المجودة حالياً في الأسواق على منتجات تحتوي على فورمالدهايد من أصول متحجرة (مواد بترولية). ومن المعروف أن انبعاث الفورمالدهايد من هذه المواد اللاصقة مضر للبيئة ويشك أنه يسبب السرطان. إضافة لذلك فإن أسعار الزيوت الباهظة وغير المستقرة، والرغبة في استعمال مواد كيماوية ذات أصل نباتي حفز البحث عن مواد لاصقة أكثر رفقاً في البيئة.
إن المصادر البيولوجية والمصادر الطبيعية الأخرى متوفرة بكثرة ويمكن أن تكون نقطة انطلاق جيدة لإنتاج مواد لاصقة خشبية رخيصة التكاليف وقابلة للتحلل ومتجددة. وخير مثال على ذلك هو استعمال البروتين والكازاين والكاربوهايدرات الخ ... ولسوء الحظ فإن لواصق الخشب المنتجة من المصادر الطبيعية لها سيئة عند مقارنتها باللواصق التقليدية وهي ضعف قوتها وضعف مقاومتها للماء وحساسيتها حين تتعرض للعفونة. وقد تم حديثاً إنتاج لواصق مكوناتها من البوليكتونات الخالية من الفورمالدهايد ذات صفات مميزة . وقد استعملت هذه اللواصق الجديدة على شكل معلقات مائية حيث تم انتاجها بخطوتين فقط.
الخطوة الأولى يتم تحضير البوليميرك أماين (polymeric amines) من تفاعل البولي كيتون غير المحسن مع ثنائي الأماين (diamines) باستعمال طريقة تفاعل بال كنور. وفي الخطوة الثانية يضاف كمية إضافية من البولي كيتون إلى الماء حيث يقوم البوليميرك أماين (polymeric amines) بدور المنشط السطحي (surfactant) في المحلول المعلق. ولكن هناك سيئة عند استعمال اللواصق المبنية على أصل البولي كيتون/أماين وهي النقص في مرونتها عند تشكلها النهائي وكذلك النقص في توفر البوليكيتون (الهامش الضيق للمواد الصلبة المتوفرة التي تحافظ على ثبات المحلول المعلق).
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أما الحسنة الرئيسية لمشتقات البوليكيتون/أماين/بروتين هي كلفتها المنخفضة مقارنة بالمشتقات المحتوية فقط على بولي كيتون/أماين، إضافة إلى ذلك فالمحلول المعلق أكثر ثباتاً.

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List of publications

1) **A.I.Hamarneh**, Y.Zhang, A.A.Broekhuis, & F.Picchioni, "Development of wood-adhesives from chemically modified polyketones", 8th World Congress of Chemical Engineering, August 23rd-27th, 2009, Montreal, Canada.

2) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis, K.A.Sjollema, Y.Zhang & F.Picchioni, "Development of wood adhesives from Soy proteins and chemically modified polyketones", *Netherlands Process Technology Symposium: Towards a world without oil*, NPS-8, October 27-29, 2008, Veldhoven, the Netherlands.

3) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis, K.A.Sjollema, Y.Zhang & F.Picchioni, "Use of soy proteins in polyketone-based wood adhesives", submitted to the International Journal of Adhesion and Adhesives.

4) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis & F.Picchioni, "Extraction of *Jatropha* curcas proteins and application in polyketone-based wood adhesives", submitted to the International Journal of Adhesion and Adhesives.

5) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis, V.de.Beus & F.Picchioni, "Use of soy proteins in polyketone-based wood adhesives: Mechanistic insight", to be submitted.

6) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis & F.Picchioni, "Wood adhesives based on natural sources: history and development: a review", in preparation.

7) **A.I.Hamarneh**, H.J.Heeres, A.A.Broekhuis, J.van.der.Velde, T.Fernandez-Landaluce, P.Rudolf & F.Picchioni, "Reactions of aliphatic polyketones with natural amino acids: a flexible route to chiral polymeric surfactants", in preparation.

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